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ACADEMIC DISSERTATION

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ABSTRACT

Hypoplastic left heart syndrome (HLHS) is the most common congenital heart defect, which necessitates single ventricle–circulation. Despite advances in survival, HLHS is still associated with significant morbidity and mortality. Myocardial dysfunction is a known risk factor for morbidity and mortality in HLHS patients throughout the treatment protocol. The aetiology of myocardial dysfunction in HLHS is still poorly understood. For the assessment of myocardial function, reliable echocardiography methods are needed. Evaluation of right ventricular (RV) function in HLHS has been difficult due to the complex anatomy and morphology of the RV. Advanced methods have been used in HLHS, but they have not been validated in patient populations. The aims of this study were to evaluate advanced echocardiographic functional methods and to utilize them in the follow-up of patients with HLHS.

Methodological studies included 51 HLHS infants and children at different phases of treatment protocol who were undergoing cardiac magnetic resonance imaging (MRI) and echocardiography under the same general anaesthetic in Evelina Children's Hospital, in London, UK. Myocardial velocity, strain and strain rate were analyzed by velocity vector imaging (VVI) and fractional area change (FAC) by two automated and one manual method and these measurements were compared to the MRI derived ejection fraction (EF). Intraobserver and interobserver repeatability was good for all techniques except the manual technique. All parameters had good correlation with MRI derived EF. FAC measured by automated techniques were best predictors of MRI EF. There was a measurement bias and wide limits of agreement between different automatic methods and therefore, single automatic method should be used.

In the clinical retrospective follow-up studies, VVI was used to assess myocardial function during the treatment protocol in a population-based cohort of HLHS neonates, infants and children (n=66) born between 2003 and 2010 in Finland. Myocardial function and clinical condition were evaluated at four phases: before stage 1, 2 and 3 operations and 0.5–2 years after stage 3 operation. Prenatal diagnosis of HLHS before stage 1 was associated with improved postnatal RV function, reduced metabolic acidosis and end-organ dysfunction compared to neonates with postnatal diagnosis. During treatment protocol, infants palliated with a Blalock-Taussig shunt (BT shunt) at stage 1 operation had higher RV volumes and more tricuspid valve regurgitation before stage 2 operation than infants that were being palliated with right ventricle to the pulmonary artery conduit (RV–PA conduit). Myocardial function of BT shunt group improved after stage 2 with better systolic performance after stage 3 as compared to those initially palliated with an RV–PA conduit.

Conclusion: VVI is a useful tool in the assessment of myocardial function throughout the treatment protocol for HLHS and comparable with MRI.

Automated methods improve the reliability of FAC measurements as compared to the manual method, but limits of agreement between these methods remain wide and therefore, different methods can not be used interchangeably. Prenatal diagnosis of HLHS is associated with improved postnatal myocardial function. The shunt type during stage 1 operation affects the long-term myocardial function. Patients palliated with a BT shunt have larger RV volume before stage 2 operation reflecting bigger volume overload but better systolic function after stage 3 operation as compared to those palliated with an RV-PA conduit.

TIIVISTELMÄ

Vasemman kammion vajaakehittyneisyysoireyhtymä (HLHS) on yleisin yksikammiolinjalla hoidettava synnynnäinen sydänvika. Vaikka selviytyminen on parantunut viime aikoina, HLHS on merkittävä syy kuolleisuudelle ja sairastavuudelle. Sydänlihaksen heikko toiminta on sairastavuuden ja kuolleisuuden riskitekijä HLHS-potilailla eri vaiheissa. Sydänlihaksen heikon toiminnan syitä tunnetaan edelleen huonosti näillä potilailla. Sydänlihaksen toiminnan tutkimiseen tarvitaan ultraäänipohjaisia menetelmiä. Oikean kammion toiminnan arviointi HLHS-potilailla on vaikeaa, koska oikean kammion anatomia ja morfologia ovat moninmutkaiset. Uusia ultaäänipohjaisia menetelmiä on kehitetty viime vuosina ja niitä on myös käytetty HLHS-potilailla siitä huolimatta, että menetelmiä ei ole validoitu HLHS:ssa. Tämän tutkimuksen tarkoituksena oli tutkia uusien ultraäänipohjaisten menetelmien käytettävyyttä HLHS-potilailla ja käyttää näitä menetelmiä HLHS-potilaiden sydänlihaksen toiminnan muutosten arviointiin.

Metodologisessa tutkimuksessa 51 HLHS-lasta kirurgisen hoidon eri vaiheissa kuvannettiin sydämen magneettikuvauksella (MRI) ja ultraäänellä saman anestesian aikana (Evelina Children's hospital, Lontoo). Nopeus vektori –kuvantamisella (VVI) mitattiin sydänlihaksen nopeus, lyhentyminen ja lyhentymisnopeus sekä lisäksi FAC (pinta-alan prosentuaalinen muutos) mitattiin kahdella automaattisella tekniikalla sekä manuaalisella tekniikalla. Kaikki muut tekniikat, paitsi manuaalinen tekniikka, olivat hyvin toistettavia. Kaikki mittaukset korreloivat hyvin MRI:lla mitattuun EF:oon. Automaattisilla tekniikoilla mitattu FAC oli paras EF:n ennustaja. Automaattisten tekniikoiden väliset arvot olivat eri suuruisia ja vaihtelu oli suurta eli yhtä automaattista tekniikka on syytä käyttää.

Kliinisessä retrospektiivisessa seurantatutkimuksessa VVI:ta käytettiin sydänlihaksen toiminnan arvioimiseen väestöpohjaisessa kohortissa HLHS-lapsia (n=66), jotka syntyivät Suomessa 2003-2010. Sydänlihaksen toimintaa ja vointia arvioitiin neljässä eri vaiheessa: ennen 1., 2. ja 3. vaiheen toimenpiteitä ja 0,5-2 vuotta 3. vaiheen toimenpiteen jälkeen. Ennen toimenpiteitä, lapsilla joille HLHS-diagnoosi oli tehty ennen syntymää oli parempi sydänlihaksen supistuvuus, vähemmän asidoosia ja muita elinmuutoksia kuin lapsilla joilla diagnoosi oli tehty vasta syntymän jälkeen. Ennen 2. vaiheen toimenpidettä lapsilla, joilla oli laitettu Blalock-Taussig (BT) –shuntti 1. vaiheen toimenpiteenä oli suurempi kammiokoko ja enemmän trikuspidaalivuotoja kuin lapsilla joilla 1. vaiheen toimenpiteessä oli laitettu putki oikeasta kammiosta keuhkovaltimoon (RV-PA). BT-ryhmässä sydänlihaksen toiminta parani 2. vaiheen toimenpiteen jälkeen ja myöhemmin BT-ryhmässä oli parempi sydänlihaksen toiminta kuin RV-PA-ryhmässä.

Yhteenvetona voidaan todeta, että VVI soveltuu hyvin HLHS-lasten sydänlihaksen toiminnan arviointiin ja korreloi hyvin magneettitutkimuksen

antamiin tuloksiin. Verrattuna manuaaliseen mittaukseen automatisoidut tekniikat ovat toistettavampia, mutta eri automatisoitujen tekniikoiden antamia tuloksia ei voi suoraan verrata toisiinsa. Ennen toimenpiteitä syntymää ennen diagnosoiduilla HLHS-lapsilla on parempi sydänlihaksen toiminta kuin syntymän jälkeen diagnosoiduilla. 1. Leikkauksessa käytetyllä shunttityypillä on vaikutus sydämen toimintaan. BT-shunttipotilailla on suurempi oikean kammion tilavuus ennen 2. vaiheen toimenpidettä johtuen suuremmasta tilavuuskuormituksesta ja myöhemmin, 3. vaiheen toimenpiteen jälkeen, parempi sydänlihaksen supistuvuus verrattuna RV-PA-potilaisiin.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four original publications, which are hereafter referred to in this text by their Roman numerals:

I Ruotsalainen HK, Bellsham-Revell HR, Bell AJ, Pihkala JI, Ojala TH, Simpson JM. Right ventricular systolic function in hypoplastic left heart syndrome: a comparison of velocity vector imaging and magnetic resonance imaging. *Eur Heart J Cardiovasc Imaging* 2016;17:687-692.

II Ruotsalainen HK, Bellsham-Revell HR, Bell AJ, Pihkala JI, Ojala TH, Simpson JM. Right ventricular systolic function in hypoplastic left heart syndrome: A comparison of manual and automated software to measure fractional area change. *Echocardiography* 2017;34:587-593.

III Markkanen HK, Pihkala JI, Salminen JT, Saarinen MM, Hornberger LK, Ojala TH. Prenatal diagnosis improves the postnatal cardiac function in a population-based cohort of infants with hypoplastic left heart syndrome. *J Am Soc Echocardiogr* 2013;26:1073-1079.

IV Ruotsalainen HK, Pihkala JI, Salminen JT, Hornberger LK, Sairanen H, Ojala TH. Initial shunt type at the Norwood operation impacts myocardial function in hypoplastic left heart syndrome. *Eur J Cardiothorac Surg*;. 2017;52:234-240.

ABBREVIATIONS

AA	aortic atresia
AS	aortic stenosis
BDG	bidirectional cavopulmonary anastomosis
BT	Blalock-Taussig shunt
CI	confidence interval
CIx	cardiac index
CV	coefficient of variation
DICOM	Digital Imaging and Communication in Medicine
DSI	dyssynchrony index
dP/dT	ventricular pressure rice ratio
EDP	end diastolic pressure
EDV	end diastolic volume
EF	ejection fraction
EFE	endocardial fibroelastosis
ESV	end systolic volume
FAC	fractional area change
Hb	haemoglobin
HLHS	hypoplastic left heart syndrome
ICC	intraclass correlation coefficient
ICU	intensive care unit
IVC	inferior vena cava
LA	left atrium
LV	left ventricle
MA	mitral atresia
MS	mitral stenosis
MPI	myocardial performance index
MRI	magnetic resonance imaging
PA	pulmonary artery
PLE	protein losing enteropathy
r	correlation coefficient
RA	right atrium
RV	right ventricle
RV EF	right ventricular ejection fraction
RV-PA	right ventricle to pulmonary artery
RVEDP	right ventricular end diastolic pressure
S	strain
SD	standard deviation
SR	strain rate
SVC	superior vena cava
TAPSE	tricuspid annular plane systolic excursion
TCPC	total cavopulmonary connection
TDI	tissue Doppler imaging
V	velocity
VVI	velocity-vector–imaging
2D	two-dimensional
3D	three-dimensional

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1. INTRODUCTION

Hypoplastic left heart syndrome (HLHS) is characterized by serious maldevelopment of the left heart structures, which necessitates univentricular circulation. It is the most common univentricular heart defect, with a prevalence of 1.6–3.6 per 10000 live births. Operative treatment was established in the1980s and consists of three operations during the first years of life, which culminates in the so-called Fontan circulation. Since the 1980s, there has been rapid improvement in survival of these patients. However, HLHS is still the most common cause of cardiac death during the first week of life and it is associated with significant morbidity and mortality throughout the treatment protocol.

Myocardial dysfunction is a known risk factor for mortality and morbidity. The aetiology of myocardial dysfunction in HLHS is still poorly understood. During the staged treatment protocol, the right ventricle (RV) meets many challenges in the adaptation to singe systemic ventricle.

Assessment of RV function in HLHS is still mainly based on subjective echocardiographic analysis, because complex RV morphology and geometry makes qualitative analysis difficult to achieve. Reliability of subjective analysis in HLHS has been shown to be poor. Magnetic resonance imaging (MRI) is the non-invasive “golden standard” for qualitative assessment of RV function in HLHS, but the availability and need for anaesthesia in small children limits its use in serial follow-up. In recent years, many echocardiography-based modalities have been developed and also used in HLHS to assess myocardial function, although these methods are not validated for the systemic RV. One of these methods is velocity-vector-imaging (VVI), which is based on speckle and contour tracking.

In this thesis, the usefulness of echocardiography–based methods in the assessment of RV myocardial function in HLHS patients has been studied and these methods have also been used to investigate myocardial function throughout treatment protocol in a population based cohort of HLHS children born in Finland between 2003–2010.

2 REVIEW OF THE LITERATURE

2.1 HYPOPOLASTIC LEFT HEART SYNDROME

HLHS is defined as a maldevelopment of the left heart structures that necessitates single ventricle circulation (Figure 1). It is associated with hypoplasia or atresia of the left ventricle (LV), mitral and aortic valves and the aortic arch. The prevalence of HLHS is approximately 1.6–3.6 per 10000 live births (Leirgul 2014, Morris 1990) and in Finland prevalence of univentricular heart is 4.4–8.9 per 10000 live births (Ojala 2013). It is more common in males (55–67%) (Morris 1990).

Without treatment, 90% of children with HLHS die before 30 days of age (Morris 1990). Surgical treatment for HLHS was established in the 1980s. The palliative surgical program for HLHS consists of three operations that ultimately culminate in the Fontan operation, which is performed as total cavopulmonary connection (TCPC). Since the introduction of the operative treatment protocol (Norwood 1992), the number of survivors has been increasing (Fixler 2010, Mahle 2000, Tibballs 2007, Tweddell 2002), but HLHS is still the most common cause of cardiac death during the first week of life (Morris 1990) and associated with significant morbidity and mortality throughout the treatment protocol.

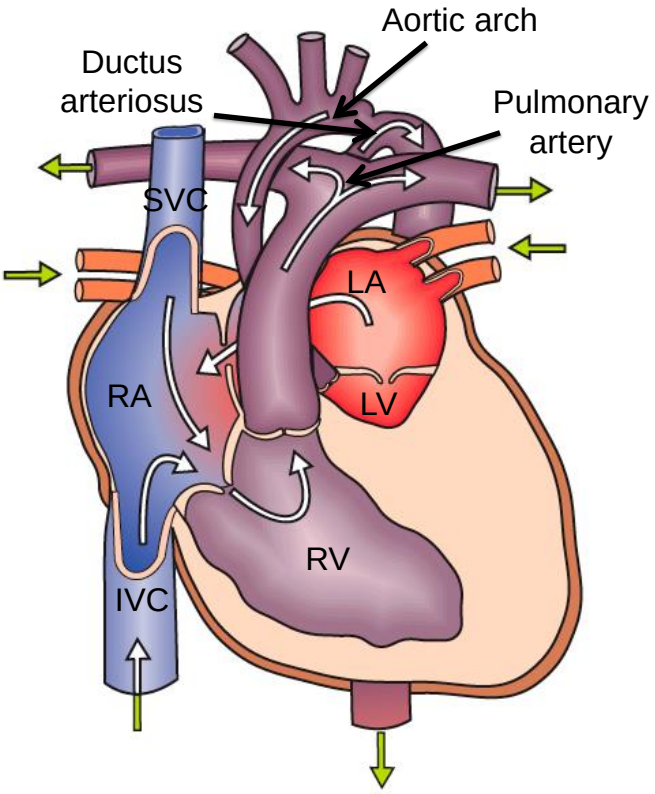

Figure 1. Anatomy of HLHS. SVC = superior vena cava, IVC = inferior vena cava, RA = right atrium, RV = right ventricle, LA = left atrium, LV = left ventricle. Modified from Sarkola 2017.

2.1.1 ANATOMICAL VARIATIONS

Anatomical variations in the size and geometry of the LV and the size of the aorta are large (Wisler 2008). The morphology of mitral and aortic valves also varies from hypoplasia and stenosis to atresia.

HLHS morphology can be divided into three groups according to the morphology of the mitral and aortic valves: mitral stenosis and aortic stenosis (MS/AS), mitral stenosis and aortic atresia (MS/AA) and mitral and aortic atresia (MA/AA). In some classifications, there is also a fourth morphology group: mitral atresia and aortic stenosis (MA/AS) in a context of a ventricular septal defect. In some but not all studies, AA has been associated with poor prognosis (Azakie 2001, Gaynor 2002). HLHS can also be classified based on the morphology of the LV that is: slit or none LV, borderline LV and globular LV.

2.1.2 MECHANISMS OF THE DEVELOPMENT OF HLHS

The aetiology of HLHS is still largely unknown. Altered haemodynamics during foetal life play a role in the development of LV hypoplasia, but the aetiology of these changes is still unknown. There is some evidence that there is a genetic component, but the inheritance seems to be multifactorial and has been associated to multiple loci and genes (Hinton 2007, Hinton 2009). There are two different suspected theories of hereditary aetiology: structural and myocardial theories.

According to the structural theory, HLHS is primarily a severe form of structural deformation of the left sided valves, which leads to altered flow conditions during foetal life and thereby to maldevelopment of the LV (Hinton 2007, Hinton 2009). High incidence of valve dysplasia in HLHS patients and siblings of HLHS patients supports this theory (Hinton 2007).There is significant variation in the severity of the maldevelopment of the left heart structures that range from a bicuspid aortic valve to HLHS and there is also strong associations between HLHS and other left sided obstructive lesions and bicuspid aortic valve (Hinton 2007, Hinton 2009). The risk of a bicuspid aortic valve in siblings of HLHS patients is 8%, which is the same as that found for siblings of patients with bicuspid aortic valves (Hinton 2007, Hinton 2009).

The other suspected theory is a primary myocardial problem, which leads to the maldevelopment of the LV with altered haemodynamics and to the maldevelopment of the left sided valves and the aortic arch. AS diagnosed during foetal life can progress to HLHS and it has been shown that intrauterine balloon dilatation of critical AS can prevent the development of HLHS. However, there is still progression of LV hypoplasia, which leads to the suspicion that there is an intrinsic pathology of the myocardium in some foetuses, after successful treatment of AS (Makikallio 2006). This speculation is supported by the finding that in foetuses with AS diagnosed in mid-gestation, LV dysfunction but not the length of LV has been one of the

predictors of development of HLHS (Makikallio 2006). Disease progression is characterized by dramatic morphological changes of the affected LV. Initially, the LV appears normal in size but with decreased contractility; then, it dilates with hyperechogenic endocardium, which is indicative of endocardial fibroelastosis (EFE) and then later in gestation, it progresses into a hypocontractile state with LV hypoplasia that meets the diagnostic criteria for HLHS (Shimada 2015). The myocardium in HLHS patients differs from that of healthy hearts, therefore this may play a causative role in the development of left ventricular hypoplasia and/or myocardial dysfunction (Salih 2004).

2.2 PRENATAL DIAGNOSIS AND OUTCOME

HLHS is one of the most common severe congenital cardiac defects diagnosed prenatally. HLHS in the foetus can be identified using four chamber screening (Altmann 2000, Brackley 2000, Tibballs 2007, Tibballs and Cantwell-Bartl 2008). There is a significant variation in the prenatal detection rate of HLHS between different centres - It was 59–85% ($p<0.001$) in single ventricle reconstruction trial centers in North America (Atz 2010) and 77% in a study from Australia (Sivarajan 2009) and 87% in Finland (Ojala 2013).The median gestational age during prenatal diagnosis is 23 weeks (Brackley 2000). Recent studies have highlighted the possibility of later foetal development of HLHS that could explain the occurrence of neonatal HLHS cases in whom the routine 19-week foetal scan failed to detect the defect (Hornberger 1995, Makikallio 2006, Marshall 2005, Tworetzky 2004). Foetal mortality has been described, but most pregnancies reach full term gestation with relatively normal growth and development of other organ systems (Galindo 2009).

Prenatal diagnosis of HLHS enables planning of the delivery with optimized postnatal stabilization of newborn babies. Ductal patency is achieved by prostaglandin treatment and neonatal transportation is avoided by transferring the mother to a suitable facility before she goes into labour. Prenatally diagnosed infants are haemodynamically more stable. They therefore require fewer and less vasoactive medications and ventilator treatmen and have lower incidences of multi-organ failure (Kipps 2011, Kumar 1999, Satomi 1999, Sivarajan 2009, Tworetzky 2001) (Table 1). Moreover, myocardial function seems to be better in prenatally diagnosed infants and they also have less tricuspid valve regurgitation (Kipps 2011, Tworetzky 2001). Additionally, children with prenatal diagnosis have fewer adverse perioperative neurological events (Mahle 2001a).

Thakur and coworkers found in a systemic review that (Thakur 2016) although prenatal diagnosis was associated with improved preoperative haemodynamics, it was not associated with better preoperative or operative survival. This may be related to there being a small number of patients (Atz 2010, Kipps 2011, Kumar 1999, Mahle 2001a, Satomi 1999, Sivarajan

2009). In one report, prenatal diagnosis of HLHS was associated with improved survival after first-stage palliation in comparison with patients diagnosed after birth (Tworetzky 2001). The prenatally diagnosed children in that same study were less likely to undergo surgery than postnatally diagnosed children and therefore, benefits of survival in this study may present a selection bias with prenatally diagnosed children with poor prognosis being less likely to undergo operative treatment (Tworetzky 2001). When the location of the delivery hospital and the length of transportation are included in the analysis, children with HLHS born far from the surgical center and without prenatal diagnosis have been shown to have increased mortality (Morris 2014).

2.3 SURGICAL TREATMENT PROTOCOL FOR HLHS

2.3.1 STAGE 1 OPERATION: THE NORWOOD PROCEDURE

Neonates with HLHS are treated with prostaglandin infusion before stage 1 operation to guarantee the patency of the arterial duct. They generally undergo stage 1 operation during the first weeks of life. The purpose of the operation is to relieve the systemic outflow obstruction, provide coronary blood flow and adequate pulmonary blood flow and create nonrestrictive atrial septal defect.

There are two different surgical options for stage I operation in HLHS (Figure 2). In the classical Norwood operation, pulmonary blood flow is provided by a Blalock-Taussig shunt (BT shunt) which connects the innominate or subclavian artery to the pulmonary artery (PA) (Norwood 1992). Alternatively, pulmonary blood flow can be provided by a RV to PA (RV-PA) conduit, the so called Sano shunt (Sano 2003). In both above mentioned types of Norwood procedure, the aortic arch is reconstructed by connecting the PA to the aorta and a nonrestrictive atrial septum is created by atrial septectomy (Norwood 1992). Finally, in some centres, the Norwood operation has been replaced by a hybrid procedure in which the interventional cardiologist stents the patent ductus arteriosus and dilates the atrial septal defect percutaneously and the surgeon performs banding of the PA branches (Gibbs 1993).

Risk factors for stage 1 operation irrespective of the type of the procedure include small weight, prematurity, restriction of the atrial septum, myocardial dysfunction and tricuspid valve regurgitation (Attar 2012, Azakie 2001, Gaynor 2002, Gelehrter 2011, Ghanayem 2012, Murtuza 2012, Photiadis 2012, Sano 2009, Simsic 2005, Tweddell 2012). There are potential advantages and disadvantages of both shunt types (BT shunt and the RV-PA conduit). In the BT shunt, there is continuous flow from the aorta to the PAs both in systole and in diastole, which causes lower systemic diastolic blood pressure, which may result in decreased myocardial perfusion and “coronary steal” (Ohye 2004). In patients with an RV-PA conduit, diastolic runoff and

coronary artery steal is avoided, but the ventriculotomy scar may add to the risk of myocardial injury and dysfunction along with arrhythmias. The main advantage of the hybrid procedure (Gibbs 1993) is the avoidance of cardiopulmonary bypass surgery in the neonatal period but after this procedure, the stage 2 operation is more challenging.

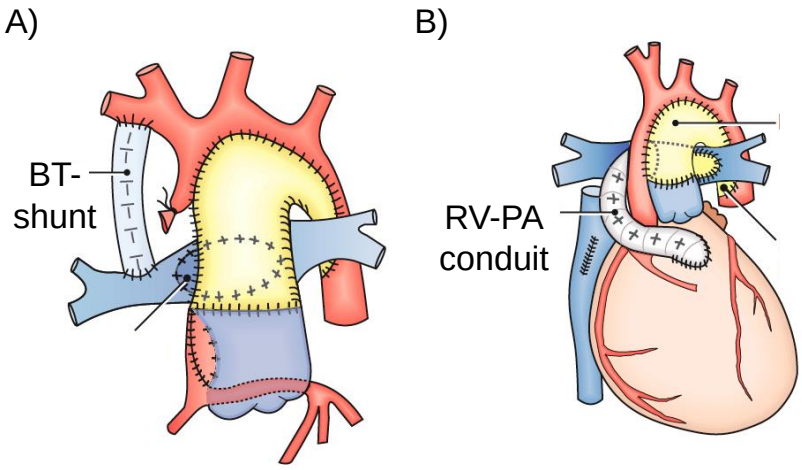

Figure 2. Surgical options for stage 1 operation in HLHS. A) Norwood operation with a BT shunt, B) Norwood operation with an RV-PA conduit (Sano shunt). Modified from Salminen et Sairanen 2017.

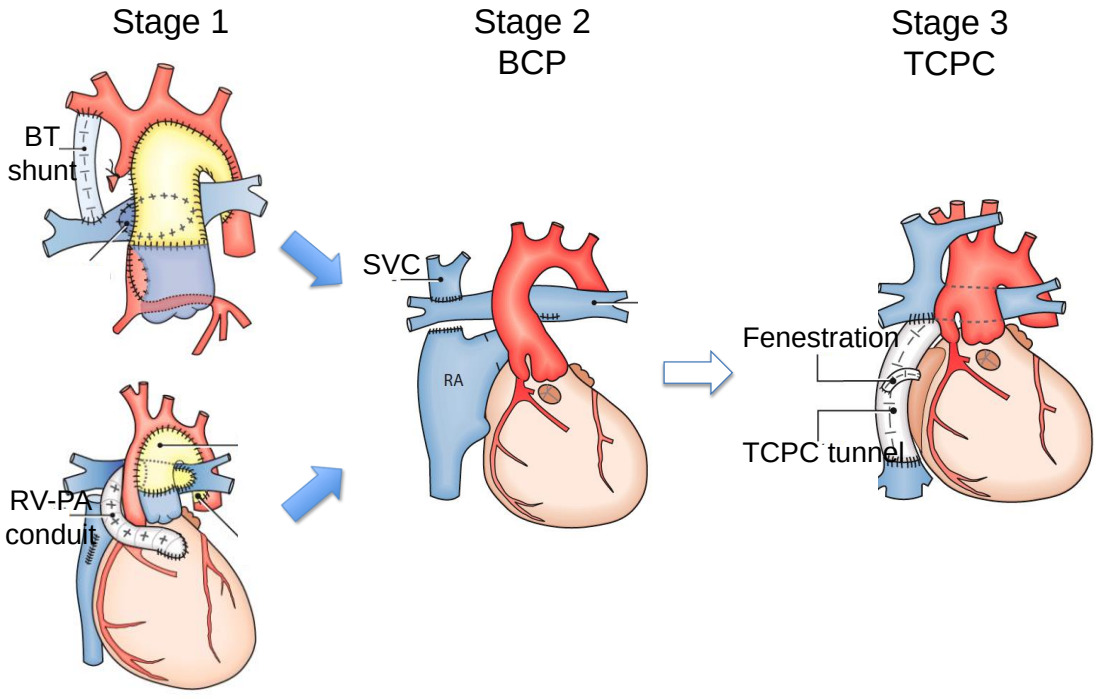

Figure 3. Staged palliation of HLHS. Modified from Salminen et Sairanen 2017.

Children with RV-PA conduit between stage 1 and 2 have more stable and efficient circulation, which manifests as improvements in clinical status and survival (Ghanayem 2012, Maher 2003, Mahle 2003, Mair 2003, Malec 2003, Pizarro 2003, Pizarro 2004, Sano 2004, Silva 2007). Small published series showed that the hybrid procedure had no impact on clinical condition or on mortality (Baba 2012, Chetan 2013, Knirsch 2012).

2.3.2 STAGE 2 OPERATION: BIDIRECTIONAL CAVOPULMONARY ANASTOMOSIS

The stage 2 operation is usually performed at the age of 4–6 months, the high-pressure source of pulmonary blood flow is eliminated and the SVC is connected to the pulmonary artery (Figure 3). This results into more efficient systemic circulation: reduced pressure and volume load for RV; usually higher level of arterial oxygen saturation and better growth potential for the infant. The two different methods for stage 2 operation are the bidirectional cavopulmonary anastomosis (BCP) and the so-called hemi-Fontan operation. There is a direct anastomosis of the SVC to the pulmonary artery in BCP. The SVC remains connected to the right atrium and the junction is closed with a patch in the semi-Fontan procedure and there is a side-to-side anastomosis of the SVC and the PA. There is no evidence that the type of stage 2 operation has a significant impact on late outcomes for patients with HLHS.

Preoperative evaluation before stage 2 operation is performed with cardiac catheterization, with cardiac MRI or with a combination of both. Evaluation consists of assessment of aortic arch, PAs, PA pressure, myocardial function and collateral circulation. Requirements for stage 2 operation are adequate sized pulmonary arteries and normal pulmonary vascular resistance. Survival from stage 2 operation is over 95%. During postoperative recovery, the most common problems are related to inadequate pulmonary circulation due to elevated pulmonary artery pressure or small pulmonary arteries and pleural effusion. Most unintended operations after stage 2 are related to correcting pulmonary circulation and the tricuspid valve.

After stage 2 palliation, RV unloading promotes the remodeling of the RV but does not decrease the degree of tricuspid valve regurgitation (Kasnar-Samprec 2012). Risk factors for mortality or transplantation after stage 2 palliation are as follows: myocardial dysfunction, tricuspid valve regurgitation, stage 2 palliation under the age of 3 months and prolonged hospitalization after stage 1 operation (Friedman 2011).

2.3.3 STAGE 3 OPERATION: TOTAL CAVOPULMONARY CONNECTION

At the stage 3 operation, the so-called Fontan circulation is completed. The IVC is connected to the PA by a synthetic tube (Figure 3). Fenestration is sometimes created between the tunnel and the atrium. Fenestration may be associated with more stable postoperative course, but it causes persistence

of systemic desaturation. Preoperative evaluation for the TCPC usually consists of haemodynamic measurements and imaging of PAs, collateral circulation, aortic arch and myocardial function by echocardiography and cardiac catheterization and/or MRI.

During postoperative recovery, most common problems are related to inadequate pulmonary circulation due to elevated PA pressure or small PAs, pleural and visceral effusions and rhythm disorders. After stage 3, additional procedures are most commonly related to PAs, aortic arch, tricuspid valve or collateral circulation. Late complications include myocardial dysfunction, rhythm disorders, tricuspid valve regurgitation, protein losing enteropathy (PLE), plastic bronchitis and failure of the Fontan circulation. Fenestration may close spontaneously or it can be closed percutaneously when the patient passes fenestration test closure. There are contradictory results, opinions about benefits and disadvantages of fenestration in HLHS patients.

2.3.4 LONG TERM OUTCOME IN PATIENTS WITH DIFFERENT INITIAL SHUNT TYPES

The RV-PA conduit provides more stable haemodynamics after stage 1 operation and this is associated with improved early survival after stage 1 operation. However, advantages in survival seem to disappear after first year after stage 1 operation and there is some evidence that long-term transplantation-free survival is better in the BT shunt group. This has raised a question about late sequelae that ventriculotomy has on myocardial function in patients with an RV-PA conduit, and the impact of shunt type on the the growth of PAs, tricuspid valve function and the need for additional procedures. There are several small published studies that compared these outcomes, but only one adequate sized randomized study has hitherto been published (Tables 2 and 3).

Reports concerning the impact of shunt type on the need for additional interventions are contradictory (Table 3). Some studies reported no difference detected in the number of interventions (Fischbach 2013). However, other studies reported that children with RV-PA conduits had more interventions and complications than those with BT shunts (Ballweg 2007, Ohye 2010, Photiadis 2012, Scheurer 2008, Tabbutt 2005). Unintended operations in the RV-PA conduit group are mostly related to PAs (Bautista-Hernandez 2011, Ohye 2010, Scheurer 2008).

Table 2. Studies comparing long-term survival in patients treated initially with either a BT shunt or an RV-PA conduit.

Study	Patients (Number of patients, study years, place)	Mortality
Bautista-Hernandez (2011)	BT/RV-PA n=82/36, Fontan 2001–2007, Boston	No difference in stage 3 survival
Graham (2010)	BT/RV-PA n=35/41, 2000–2005, South Carolina	No difference at 6.8 years
Lai, Scheurer (2007, 2008)	BT/RV-PA n=27/29, 2002–2003, Boston	No difference in survival after stage 2, until the age of 3 years
Photiadis, Fischbacb (2012, 2013)	BT/RV-PA n=71/38, 2002–2009, Germany	No difference until 4.1 years after stage 1
Pizarro (2004)	Stage I hospital survivors: BT/RV-PA n=46/50	Better survival in RV-PA group until stage 2
Polimenakos (2014)	BT/RV-PA n=26/28, 2005–2010, USA	No difference until 39.6 months
Silva (2007)	Stage I patients: BT/RV-PA n= 37/34, 1999–2006	Better survival in RV-PA group until stage 2
Single ventricle reconstruction trial (Ohye 2010)	BT/RV-PA n=275/274, Randomized, multicentre,	Better survival in RV-PA group at 12 months (74% vs. 64%, p=0.010), no difference after that until 4.8 years
Wilder (2015)	BT/RV-PA n=169/169, 2005–2014, multicentre	6-year survival better in RV-PA group (70% vs. 55%, p<0.001)

Table 3. Studies comparing the impact of the initial shunt type on pulmonary arteries, aortic arch, additional procedures and myocardial function. TI tricuspid insufficiency, PA pulmonary arteries, NO no difference.

Study	Patients (n, years, place)	Follow-up	PA	TI	Morbidity	Myocardial function
Before stage 2						
Single ventricle reconstruction trial (Ohye 2010, Frommelt 2014)	BT/RV-PA n=275/274, Randomized, multicentre, North America	4.8 years	NO		At 12 months more interventions and complications in RV-PA	Better EF in RV-PA.
Wilder (2015)	BT/RV-PA n=169/169, 2005–2014, multicenter	6 years.		NO	NO	More dysfunction in BT.
Fischbach, Photiadis (2012, 2013)	BT/RV-PA n=70/37, 2002–2009, Germany	3.5 years after stage 1	NO		More shunt related and aortic arch intervention in RV-PA	
Before stage 3						
Lai, Scheuer (2008)	BT/RV-PA n=27/29, 2002–2003, Boston	Until stage 3	NO		NO	
Januszewska (2007)	BT/RV-PA n=19/31, stage 3 1995–2006, Poland	During stage 3		NO	NO	NO
Polimenakos (2014)	BT/RV-PA n=26/28, 2005–2010, USA	39.6 months after stage 1			No difference on interventions	NO
Graham (2010)	BT/RV-PA n=35/41, 2000–2005, South Carolina	6.8 years after stage 1	Better growth of PAs in RV-PA	NO		Poorer function in RV-PA
Single ventricle reconstruction trial (Ohye 2010, Frommelt 2014)	BT/RV-PA n=275/274, Randomized, multicentre, North America	4.8 years	NO		More interventions and complications in RV-PA	NO at 14 months. Before stage 3 lower function in RV-PA.
After stage 3						
Bautista-Hernandez (2011)	BT/RV-PA n=82/36, Fontan 2001–2007, Boston	28.4 months after stage 3	More intervention in RV-PA	More interventions in BT		NO
Wilder (2015)	BT/RV-PA n=169/169, 2005–2014, multicentre	6 years.		NO	NO	NO

2.4 MYOCARDIAL FUNCTION IN HLHS

2.4.1 MYOCARDIAL FUNCTION DURING FOETAL LIFE

Adaptation of the RV in HLHS starts during foetal life. The RV volume, stroke volume, and cardiac output in HLHS foetuses may be increased compared with those of the gestational age-matched normal controls (Jiang 2016, Szwast 2009). However, the total cardiac output has been shown to be 20% smaller than in healthy foetuses (Szwast 2009). The foetal RV in HLHS becomes more spherical because of increased RV diameter. It has relatively reduced longitudinal contraction as compared with circumferential contraction and an increased reliance on atrial contraction for ventricular filling during diastole (Axt-Fliedner 2015, Brooks 2012, Graupner 2016, Miller 2012, Vyas 2011). The amount and composition of extracellular matrix both in RV and residual LV in HLHS foetuses differs from healthy foetuses (Salih 2004). This may present inherent abnormality and have significant implications to myocardial function in patients with HLHS (Salih 2004).

In summary, foetuses with HLHS have preserved RV systolic performance but impaired diastolic performance as compared with normal foetuses. The heart of a foetus with HLHS is less efficient than the normal heart in that the ejection force of the RV is increased, but overall delivery of cardiac output is lower than that of a normal heart.

2.4.2 MYOCARDIAL FUNCTION DURING THE OPERATIVE PROTOCOL FOR HLHS

After birth, the adaptation to postnatal circulation is challenging for the RV myocardium of HLHS neonates. There is a rapid increase in the systemic vascular resistance and total cardiac output. Haemodynamic instability may affect coronary circulation and lead to myocardial ischaemia. Prenatal diagnosis is associated with improved haemodynamic stability (Thakur 2016). There are contradictory results whether a prenatal diagnosis has an impact on myocardial function postnatally (Kipps 2011, Petko 2011a). This may be related to differences in methods used for myocardial functional analysis.

The volume load of RV increases after stage 1 operation. Increased volume load and cardiopulmonary bypass during stage 1 operation lead to diminished systolic function in early postoperative period, but there is some improvement before stage 2 operation (Christensen 2006, Saiki 2016). There is often a slight increase in RV size (Marx 2013). After stage 1 operation, longitudinal contraction, the main contraction pattern in a normal RV, diminishes and radial contraction increases. The contraction pattern changes to a more left ventricle–like pattern. If this adaptation of the contraction pattern fails, there is more dyssynchrony and deterioration of systolic

function.

During the interstage period between stages 1 and 2, patients with BT shunts have larger RV volumes both in systole and diastole and lower ejection fraction (EF) and contractility than in patients with RV-PA conduits (Frommelt 2012, Hughes 2004, Ohye 2010, Wilder 2015). These differences are probably related to higher volume load of RV. Lower diastolic blood pressure may also have an impact through lower coronary perfusion pressure and myocardial ischaemia. There are also conflicting results from small patient series regarding the effect of shunt type on myocardial function (Ballweg 2007, Graham 2007, Photiadis 2005). These differences are probably related to operative techniques, selection bias and the small number of patients in addition to the different methods used to assess myocardial function. There is reduced contractility at the site of the ventriculotomy in patients treated with an RV-PA conduit, and there is also a greater remodeling of the ventricular myocardial extracellular matrix as compared to those treated with a BT shunt (Menon 2011, Menon 2013). This may cause suboptimal ventricular performance later during the treatment protocol (Padalino 2008).

The RV volume load decreases after stage 2 operation. The end-diastolic volume (EDV) and end-systolic volume (ESV) decrease, stroke volume does not change and EF increases (Bellsham-Revell 2013b, Seliem 1993). Adaptation to more LV–like contraction pattern usually continues. This adaptation of RV fails in some patients, but risk factors for that event are still largely unknown (Bellsham-Revell 2013b). Maladaptation is associated with tricuspid valve regurgitation and collateral vessels before stage 2 operation. The change in RV volume is bigger in patients with borderline LV morphology (Bellsham-Revell 2013b).

During the interstage period between stages 2 and 3, the difference in RV volume and EF between shunt types disappears. In the single ventricle reconstruction trial that compared shunt types, the difference in EF was no longer apparent at the age of 14 months, and before stage 3 operation, children in the RV-PA group have lower RV function than BT group (Frommelt 2012, Frommelt 2014, Hill 2015). Lower myocardial function in patients with RV-PA conduits is seen in other studies (Ballweg 2010, Graham 2010). This difference might be associated with long-term consequences of myocardial scar. Some studies have failed to demonstrate differences in myocardial function between shunt types (Januszewska 2007, Polimenakos 2014).

There is a further decrease in RV volume load after the stage 3 operation. However, no marked changes are seen in RV volume or function. There are only a few studies that have investigated long-term myocardial function in patients with HLHS after Fontan completion. Myocardial function was shown to deteriorate in some studies, but the risk factors for that phenomenon are largely unknown. There are only a few studies that compare the impact of initial shunt type on myocardial function after stage 3 operation. No difference was seen at six years after stage 1 in a multicentre study that compared myocardial function of propensity-score matching paired 169

children with initial BT shunts with 169 children with RV-PA conduits. The incidence of late myocardial dysfunction was less than 5% (Bautista-Hernandez 2011, Wilder 2015).

Myocardial dysfunction is a risk factor for mortality and transplantation during treatment protocol for Fontan circulation (Altmann 2000, Chetan 2013, Friedman 2011, Hughes 2011, Jean-St-Michel 2016, Simsic 2005, Tweddell 2012, Walsh 2009). However, there is a recovery of RV dysfunction after stage 2 in most children with RV dysfunction after stage 1 operation (O'Connor 2012). Myocardial dysfunction is associated with congestive heart failure, arrhythmias and PLE after stage 3 operation (Kotani 2009). Myocardial dysfunction, mechanical dyssynchrony and asymmetric contraction are associated with moderate and severe tricuspid valve regurgitation regardless of surgical strategy (Bharucha 2013, Chetan 2013).

Mechanisms for myocardial dysfunction in HLHS include a maladaptive contraction pattern, myocardial ischaemia, dyssynchrony and tricuspid valve regurgitation. HLHS morphology and residual LV size are associated with regional function and shape of the RV, but not with global function. HLHS children have more myocardial dyssynchrony as compared with healthy children but there are contradictory results as to whether myocardial synchrony is related to myocardial function in HLHS children (Friedberg 2007b, Khoo 2011).

2.4.3 TRICUSPID VALVE REGURGITATION

Tricuspid valve regurgitation in HLHS patients may be associated with structural alterations in the tricuspid valve or with myocardial dysfunction and annulus dilatation. Further, 56% of HLHS patients have been shown to have a structural malformation of the tricuspid valve apparatus (Hinton 2007) but there is a wide range of different types of structural alterations (Stamm 1997), thus: the subvalvar apparatus is different from normal in patients with MA, whereas dysplasia of leaflets occurs more often together with MS (Stamm 1997).

The incidence of tricuspid valve regurgitation in patients palliated with a BT shunt after stage 1 is higher than in those with an RV-PA conduit, but no difference is seen after that (Graham 2010, Wilder 2015).The size of the tricuspid valve annulus after unloading of RV at stage 2 operation remains unchanged in patients with significant regurgitation and decreases in the rest of the patients with no change in the grade of regurgitation (Kasnar-Samprec 2012). Surgical tricuspid valve plasty is usually successful in patients with HLHS and tricuspid valve regurgitation, and late failures are associated with myocardial dysfunction (Bove 2007). Children with a previous BT shunt seem to have more tricuspid valve operations during the course of their treatment protocol (Bautista-Hernandez 2011) than those with an RV-PA conduit.

2.5 METHODS FOR THE ASSESSMENT OF MYOCARDIAL FUNCTION IN HLHS

2.5.1 CARDIAC CATHETERIZATION

The golden standard for the assessment of RV function in HLHS is cardiac catheterization. Catheterization provides angiographic imaging and haemodynamic measurements in addition to the possibility of performing interventions for stenotic or shunt lesions when needed. It is an invasive procedure requiring general anaesthesia in children and it is not without risks, and therefore, it cannot be used in routine serial follow-up of HLHS patients. Cardiac catheterization for HLHS patients has been used in preoperative assessment before stages 2 and 3, for the assessment of surgical results and for interventional procedures such as closure of fenestration or collaterals or dilatation of aortic coarctation or pulmonary artery stenosis. Cardiac MRI has replaced cardiac catheterization in preoperative assessment in patients in many centres in recent years without the need for interventional procedures.

2.5.2 MRI

MRI, particularly the MRI-derived EF, is currently considered the noninvasive gold standard for the evaluation of RV systolic function in patients with HLHS (Mertens and Friedberg 2010). MRI is less widely available than echocardiography and it requires general anaesthesia in small children, which limits its use in serial assessment and clinical follow-up. MRI is also heavily influenced by loading conditions (Bellsham-Revell 2013b). There is good interobserver agreement for systolic and diastolic volumes, EF and stroke volume measured by MRI intraclass correlation coefficient (ICC) and 95% confidence interval (CI): EDV 0.945 (0.741–0.91), ESV 0.952 (0.779–0.984), stroke volume 0.926 (0.780–0.970) and EF 0.885 (0.764–0.946)) (Bellsham-Revell 2013b) in HLHS patients. MRI is also used for perioperative assessment of the haemodynamics and vessels sizes for those patients not needing interventional procedures listed before.

2.5.3 ECHOCARDIOGRAPHY

Echocardiography is widely used to assess myocardial function in children with HLHS. It is easily available and there is no need for general anaesthesia, which makes it very suitable for serial follow up. Several echocardiographic methods can be used to assess myocardial function but only few of them have been validated for HLHS (Table 4).

Table 4. Methodological studies comparing different echocardiography based methods to MRI or cardiac catheterization (cath) for the assessment of myocardial function in patients with HLHS

Study	Method	Parameters	Patients	Question	Results
Subjective					
Muthurangu 2005	Echo vs. MRI	Echo: qualitative; MRI: EF	N=37, before stage II		Subjective evaluation found poor function
Bellsham-Revell 2013	Echo vs. MRI	Subjective evaluation of RV function	N=28, all stages	Reliability of subective evaluation	Agreement between echo and MRI EF low 47.6%.
Conventional					
Trowitzsch 1985	Echo vs. cath.	Echo: EF, FAC; cath EF	N=12	Validation	EF and FAC correlate with cath
Friedberg 2007	Echo vs. cath.	Echo: FAC; cat: RVEDP, CIx	N=33, all stages	New index for RV function	No correlation to cath. Underestimates myocardial dysfunction.
Schlangen 2014	Echo vs. cath.	Echo: FAC, TAPSE.	N=52, after stage III	Load dependency	
Avitable 2014	Echo vs. MRI	TAPSE, MRI EF	N=29, after stage III	Usefulness of TAPSE	TAPSE reproducible, no correlation with MRI EF.
Doppler					
Michelfelder 1996	Echo vs. cath.	dP/dt	N=13 all stages	Validation of echocardiographic dPT/dt	dP/dt measured by echo reproducible and correlates with cath
Friedberg 2007	Echo vs. cath,	Echo: Systolic to diastolic duration ratio, MPI; cath: RVEDP, CIx	N=33, all stages	New index for RV function	No correlation to cath. Higher values in myocardial dysfunction.
3D					
Bell 2014	Echo vs. MRI	EF, volumes, stroke volume	N=28, all stages	Usefulness of 3D measurements	3D reproducible, correlates with MRI, lower values in echo and cannot be used inter-changeably.

Tissue doppler and speckle tracking					
Khoo 2011	Echo (speckle tracking) vs. MRI	Echo: S, SR, dyssynchrony index	N=20, before stage II	Correlation of echo parameters with MRI	S, SR, dyssynchrony index correlate with EF
Bellsham-Revell 2012	Echo (tissue doppler) vs. MRI	TDI: MPI, S/D ratio	N=57, all stages		No correlation with MRI EF
Husain 2013	Echo (tissue doppler) vs. cath.	Echo: SR diastole, TDI; cath. EDP	N=27, 11.4 months (0–132)	Usefulness of SR and TDI	SR correlates with EDP, finds EDP>10 mmHg, TDI no correlation with EDP
Schlangen 2014	Echo (sepckle tracking) vs. cath.	Echo: S, SR.	N=52, after stage III	Load dependency	SR load independent and correlates with end systolic elastance.

Conventional methods

Quantitative echocardiographic assessment of RV function in HLHS patients is challenging because of the complex anatomy and morphology of the RV. Therefore, 95% of pediatric cardiologists in North America use only qualitative echocardiography to assess RV systolic function in clinical follow-up of HLHS children at different stages of the surgical program (Nadorlik 2014). The agreement between qualitative echocardiography and MRI EF is quite low, only 47.6% (range, 31.4–58.2%) (Bellsham-Revell 2013a), and in screening of myocardial dysfunction, sensitivity of qualitative echocardiography is quite good but specificity low (Bellsham-Revell 2013a, Muthurangu 2005). Poor EF is detected relatively easily, but moderate and normal function as assessed by echocardiography includes a wide range of EFs in MRI (Muthurangu 2005). Agreement improves with experience, but remains suboptimal even in the hands of an experienced pediatric cardiologist (Bellsham-Revell 2013a) and therefore, quantitative methods are also needed to complement the qualitative methods.

Conventional quantitative methods that are used to evaluate RV systolic function in HLHS are EF, fractional area change (FAC) and tricuspid annular plane systolic excursion (TAPSE). The EF is a measure of the change in volume between diastole and systole. The assumptions about RV shape in 2-dimensional (2-D) echocardiography are mainly based on the elliptical shape of normal RV. The FAC value is a measure of the percentage of change in RV area between diastole and systole. The TAPSE value is a measure of RV longitudinal shortening, which is the main component of RV contraction in a normal RV (Brown 2011). It is defined as the excursion of tricuspid valve annulus towards the RV apex in systole (Hammarstrom 1991).

The comparisons of TAPSE, FAC and EF with cardiac catheterization and MRI measurements in HLHS patients are presented in Table 4. Echocardiographic EF and FAC measurements correlate with EF measured by cardiac catheterization (Trowitzsch 1985). Intraobserver and interobserver repeatability of these methods in HLHS patients have not been reported. FAC can be measured in 2D echocardiography by automated border detection and it has been shown to be feasible in children with HLHS (Kimball 1996). TAPSE is reproducible but did not have a correlation to EF when measured by MRI in HLHS children (Avitabile 2014). The disadvantage of all these methods is that they are load dependent in HLHS (Schlangen 2014).

Doppler

Different Doppler based methods that analyze the tricuspid valve regurgitation jet have been used to assess myocardial function in HLHS.

dP/dt of the tricuspid regurgitant jet is measured using the Bernoulli equation (Michelfelder 1996). Intraobserver and interobserver variabilities for dP/dt are small and there is good correlation with dP/dt measured in cardiac catheterization (Michelfelder 1996). This method has not been compared to EF measurements and it is unknown how dP/dt reflects myocardial function.

The systolic to diastolic duration ratio is calculated from tricuspid valve regurgitation jet data that is obtained by continuous Doppler flow measurement (Friedberg and Silverman 2007) and the ratio reflects global RV function in HLHS (Friedberg and Silverman 2007). Higher values are related to myocardial dysfunction that are measured by the FAC and qualitative method (Friedberg and Silverman 2007), but there is no correlation between systolic to diastolic duration ratio and cardiac index (CIx) or RV EDP measured in cardiac catheterization (Friedberg and Silverman 2007). Systolic to diastolic duration ratio varies depending on the stage of palliation (Friedberg and Silverman 2007).

Major limitation in these Doppler based measurements is that they cannot be performed in neonates, infants and children with no or only mild tricuspid valve regurgitation (Friedberg and Silverman 2007, Michelfelder 1996).

Three-dimensional echocardiography

The EF can be measured by using three-dimensional (3D) echocardiography of true volumes without making assumptions about the RV shape. The 3D measurements have been shown to be reproducible and to correlate with MRI measurements (Bell 2014). However, EF values are lower in magnitude in 3D echocardiography than in MRI and therefore they cannot be used inter-changeably (Bell 2014).

Tissue Doppler

Velocities of tissue motion can be measured by using the tissue Doppler imaging (TDI) method. Regional velocities are used to assess myocardial function. Indices of myocardial function such as myocardial performance index (MPI) and systolic to diastolic ratio are generated from tissue velocities. Tissue Doppler measurements do not correlate with MRI EF or with RV EDP measured in cardiac catheterization (Bellsham-Revell 2012, Husain 2013). Myocardial strain (S) and strain rate (SR) can also be measured by tissue Doppler. S is the change in length of myocardium expressed as percentage and SR is the velocity of this change. S or SR measured by TDI have not been critically evaluated in HLHS patients (Table 4). Angle dependency limits the use of tissue Doppler derived measurements.

Speckle tracking

Speckle tracking is a method in which the ultrasound speckles within a 2-D grayscale image are tracked. The S value is determined from the displacement of these speckles in relation to each other and the strain rate (SR) is the velocity of this change. This method is angle-independent. Myocardial S and SR are valuable tools in measuring regional contraction. Intraobserver and interobserver agreement with speckle tracking in HLHS ranges from acceptable to good: coefficient variation (CV) 95% confidence interval (CI) for S in diastole 0.81 (0.52–0.92), systole 0.83 (0.58–0.93) and SR in diastole 0.81 (0.52–0.92) and in systole 0.93 (0.84–0.97) (Schlangen 2014). The TDI measurements have not had a correlation with EDP measured in cardiac catheterization (Husain 2013), but diastolic SR has correlated with EDP and predicted EDP >10 mm hg with 87.5% sensitivity and 78.9% specificity (Husain 2013). Ventricular S as measured by speckle tracking has had a correlation with MRI EF before stage 2 operation ($r=0.72$, $p=0.010$), SR ($r=–0.85$, $p=0.001$) (Khoo 2011).

The influence of loading conditions on speckle tracking measurements has been investigated in patients with HLHS after stage 3 operation by comparing the parameters obtained by 2-D speckle tracking to end-systolic elastance measured in the cardiac catheterization and SR has been found to be load independent (Schlangen 2014). A decline in myocardial function may be detected earlier with the speckle tracking method than with the conventional methods (Michel 2016).

VVI

VVI is an echocardiographic method based on feature-tracking, which incorporates speckle and endocardial contour tracking (Pirat 2006). The VVI method has been validated for the LV ((Pirat 2006) and subpulmonary RV (Pirat 2006). VVI measures myocardial velocities (V), myocardial deformation (S and SR) and mechanical synchrony and is independent of the angle of

insonation. It has been used on HLHS foetuses and HLHS children to assess myocardial function and mechanical synchrony. However, the technique has not been critically evaluated or validated by comparison with MRI derived RVEF in patients with HLHS.

Mechanical dyssynchrony

Mechanical dyssynchrony in HLHS patients has been measured by TDI (Gokhale 2013, Khoo 2011, Motonaga 2012, Petko 2010, Stiver 2015), speckle tracking (Stiver 2015) and by VVI (Bharucha 2013, Friedberg 2007b, Motonaga 2012, Petko 2011b). Mechanical synchrony can be evaluated by measuring the difference in time to peak longitudinal velocity, S or SR between free and septal wall (Bharucha 2013). It can also be measured by a dyssynchrony index (DSI), which is calculated from the standard deviation (SD) of the time to peak V, S or SR between different segments (Friedberg 2007b, Khoo 2011). Adjustment for heart rate can be done either by dividing the result by the square root of the length of the cardiac cycle or by using a percentage of the length of the systole (Khoo 2011).

Compared to healthy controls, the children with HLHS have mechanical dyssynchrony as measured by VVI (Friedberg 2007b) with no significant relationship between mechanical dyssynchrony and QRS duration or with FAC (Friedberg 2007b). Interestingly, mechanical DSI measured by speckle tracking has been reported to correlate linearly with MRI derived RVEF ($r=-0.73$, $p=0.010$) (Khoo 2011).

3 AIMS OF THE STUDY

The aims of this study were to evaluate the advanced echocardiographical functional methods used for HLHS patients both in methodological (I-II) and clinical (III-IV) studies. The specific study questions included:

I. Reliability of VVI measurements of RV systolic function in comparison with the non-invasive golden standard: MRI derived RV EF.

II. The repeatability of different echocardiographic based techniques, both manual and automated to measure the FAC and to correlate these measurements with the non-invasive golden standard: MRI derived RV EF.

III. The impact of prenatal diagnosis on the myocardial function and operative mortality.

IV. Determine perioperative risk factors for myocardial dysfunction in different phases of the operative treatment protocol

4 PATIENTS AND METHODS

4.1 PATIENTS

4.1.1 STUDIES I AND II (LONDON, UNITED KINGDOM)

Patients with classical HLHS who underwent routine transthoracic echocardiography and cardiac MRI under general anaesthetic at Evelina London Children's Hospital (London, UK) between July 2007 and September 2010 were included. Cardiac MRI is performed routinely in this unit before stages 2 and 3 operations. After stage 3 operation, cardiac MRI is performed only when there is clinical suspicion of complications. HLHS was defined as any combination of MS or MA with AS or AA (with atrioventricular and ventriculoarterial concordance) with no ventricular septal defects that necessitates Norwood palliation. The LV morphology was described as follows: no LV visible or slit-like LV; globular LV; or borderline LV. Borderline LV was defined as a more mildly hypoplastic LV that could be considered potentially able to support the systemic circulation. In general, the no visible LV or slit-like LV corresponded to the MA/AA subgroup, the globular LV to the MS/AA subgroup, and the borderline LV to the MS/AS subgroup. Patients were at different stages of palliation (between stages 1 and 2, between stages 2 and 3 or after stage 3 palliation).

Patients were excluded when MRI and echocardiography were not possible in the same general anaesthesia or when the quality of the four-chamber view was insufficient for manual FAC analysis. Patients with unbalanced atrioventricular septal defects, double-outlet RV, or significant ventricular septal defects were excluded. Patients with hybrid-approach as in stage 1 palliation were also excluded from study before stage 2. Ethical and institutional approval was granted for this prospective study and informed consent was obtained from parents or legal guardians before examinations were implemented.

A total of 51 patients at different stages of treatment protocol had both MRI and echocardiographic studies available for analysis (Table 5). The majority of patients had RV myocardial function within normal range as assessed by MRI (Table 5).

Table 5. Demographics of 51 children with HLHS (study I, London).

	Median (range) / Number (percentage) / Mean±SD
Age (y)	2.0 (0.1–13.7)
Male/female (n (%))	36 (71%) / 15 (29%)
Weight (kg)	9.7 (3.0–45.9)
Height (cm)	78 (49–160)
Morphology group A/B/C (n (%))	18 (35%) / 10 (20%) / 23 (45%)
Stage 1/2/3 (n (%))	23 (45%) / 22(43%) / 6 (12%)
MRI EF (%)	58.9±9.2

A = Slit/none LV, B = Borderline LV, C = Globular LV; MRI EF = ejection fraction assessed by MRI

4.1.2 STUDIES III AND IV (HELSINKI, FINLAND)

All HLHS patients (n=66) born between years 2003–2010 in Finland referred to preoperative evaluation for the Norwood operation to the Children's Hospital, Helsinki University Hospital, Helsinki, Finland were selected from an institutional surgical database and diagnosis list and these patients were included in the study (Table 6). All pediatric cardiac surgery in Finland is centralized to Helsinki and this study population represents the national cohort of consecutive patients with HLHS who underwent the Norwood procedure between January 2003 and December 2010. The study was approved by the hospital research committee and the Ethics Board.

HLHS was defined as atrioventricular and ventriculoarterial concordance with MS or MA and AS or AA combined with a small left ventricle incapable of maintaining systemic cardiac output and necessitating Norwood palliation. Patients with major extracardiac anomalies or chromosomal abnormalities were excluded from the study. Clinical characteristics of study patients are presented in Table 6. Patients were divided into three morphology groups according to the morphology and function of mitral and aortic valves at the first echocardiography: MA/AA, MS/AA and MS/AS.

In Study III, patients were divided into two groups based on whether they had prenatal or postnatal diagnoses of HLHS. The follow-up study (Study IV) included only those patients who underwent stage 1 operation (n=63). Two patients from the original study (Study III) died before operation and one underwent conversion to two chamber circulation during stage 2 operation. In Study IV, we divided the patients into 2 groups according to the type of shunt (BT shunt or RV-PA conduit) used in the Norwood procedure. Patients were examined at four time points immediately prior to surgical stages, thus: Stage 1 (Norwood operation), Stage 2 (BCP), before Stage 3 (TCPC) and at the last follow-up 0.5–3 years after stage 3. All patients were followed up until death or the end of the study period.

Table 6. Demographics of 66 HLHS neonates (Study III)

	Median (range)
Male/female (n (%))	38 (58%) / 28 (42%)
Gestational age at birth (weeks)	39+4 (36+0-42+0)
Birth weight (kg)	3.5 (2.2–4.6)
Height (cm)	78 (49–160)
Apgar score (points)	9 (6–10)
Morphology group A//B/C (n (%))	18 (35%) / 10 (20%) / 23 (45%)
Prenatal diagnosis (n (%))	25 (38%)

A: MS/AS, B: MS/AA, C: MA/AA.

4.2 CLINICAL VARIABLES

Studies III and IV

Medical records of the patients were reviewed for the demographic, anatomical and clinical characteristics and operative data. Clinical details included weight, oxygen saturation, blood pressure and cardiac medications at the time of the echocardiographic evaluation. Additionally, in Study III, the lowest arterial pH and highest lactate level were measured and kidney function was assessed through the highest preoperative creatinine and liver function through the maximum alanine aminotransferase concentrations. In Study IV; operative data included aortic cross clamp time, cardiopulmonary bypass time, duration of ventilation, length of intensive care stay, duration of pleural effusions and duration of hospitalization. Additional procedures performed at BDG and TCPC operations or during study period such as. tricuspid valvuloplasty, pulmonary artery reconstruction and aortic arch repair, were also noted. Additionally, data were collected from reports of cardiac catheterizations performed before Stages 2 and 3 and 0.5–3 years after Stage 3.

4.3 TREATMENT PROTOCOL AND SURGICAL METHODS

Studies I and II (London)

A BT shunt was used for palliation in all patients at stage 1 operation. During the study period, some high-risk patients were palliated with hybrid procedure, but these patients were excluded from the study. A hemi-Fontan procedure was performed as stage 2 operation and extracardiac TCPC as stage 3 operation.

Studies III and IV (Helsinki, Finland)

All infants with prenatal diagnosis were delivered at the University Hospital of Helsinki. Term vaginal delivery was planned and a caesarean section was used only after obstetric indications. Prostaglandin infusion was started immediately after birth before admission to the ICU. Respiratory support and

inotropes were not routinely started. Infants without prenatal diagnoses were born in delivery hospitals around Finland and were transported to Helsinki University Hospital after diagnosis and stabilization.

The operative approach for HLHS patients during the study period included a Norwood procedure during the patient's neonatal period, stage 2 operation at the age of 2–6 months during the patient's infant period and stage 3 operation at the age of 2–4 years. Hybrid operations were not performed. All operations were performed by four experienced staff surgeons.

Contraindications for stage 1 operation during the study period were: chromosomal anomalies, major extracardiac anomalies, prematurity, birth weight under 2.5 kg, severe tricuspid insufficiency and severe myocardial dysfunction (assessed qualitatively). During the study period, the type of the shunt used in the Norwood procedure (BT shunt or RV-PA conduit) was chosen according to the institutional policy, in a way that only one shunt type was performed during a specific time period and both shunt types were equally performed for high-risk patients. A BT shunt was used as the source of pulmonary blood flow between January 2003 and October 2003 and again between August 2006 and March 2010. An RV-PA conduit was used during the other time periods of the study era (Figure 4).

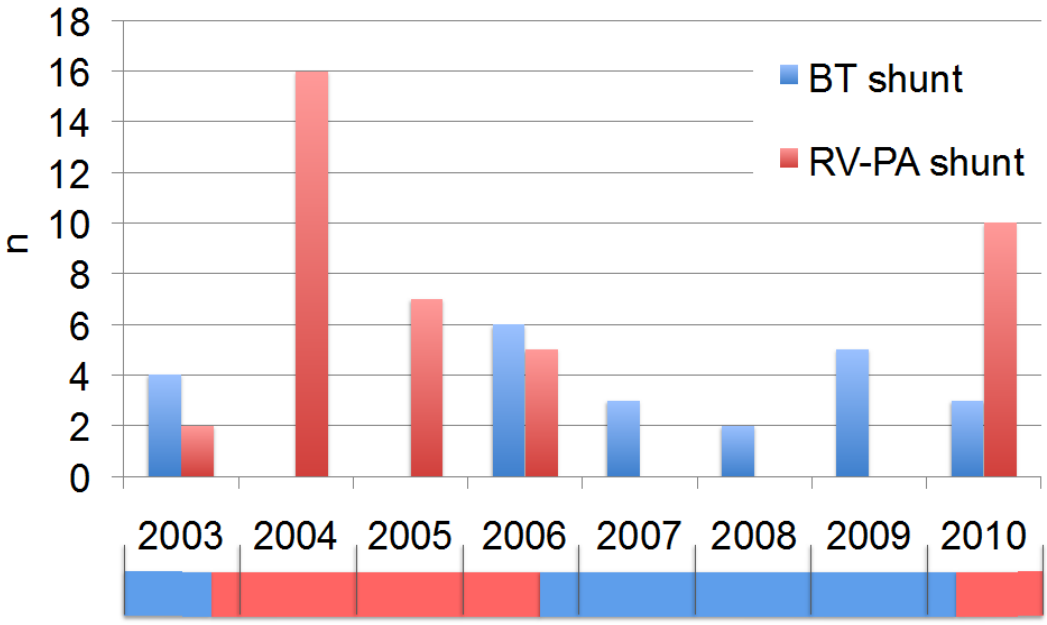

Figure 4. Type of shunt (BT or RV-PA) used in the Norwood procedure and number of patients with each shunt type during the study period (study IV)

The ventriculotomy for the RV-PA conduit was performed in the RV infundibulum. The incision was made with a knife and enlarged by retracting the muscle fibers with the tip of the scissors. The proximal end of the shunt was inserted through the ventriculotomy inside the cavity and fixed with a purse string suture and a stich. The distal end was connected to the PA with

an end-to-side anastomosis. Antegrade cerebral perfusion in moderate hypothermia (20º to 24 ºC) and cold blood cardioplegia were used in all patients.

Stage 2 operation was conducted at the age of 2–6 months. Indications for earlier operation were severe desaturation or congestive heart failure not treatable with medications. Contraindications for stage 2 operation were high pulmonary resistance and severe neurological problems. The BCP involved mobilization and transsection of the SVC to create an end to side anastomosis between the SVC and right PA. At stage 2 operation, the proximal Gore-Tex™ conduit was ligated in all patients with an RV-PA conduit. The ventriculotomy was not closed.

Contraindications for stage 3 operation were the inability to walk and severe neurological problems, high pulmonary vascular resistance, small PAs and severe myocardial dysfunction. At stage 3 operation, an 18 or 20 mm Gore-Tex™ extracardiac conduit was inserted between the IVC and the PA. Fenestration was created in all patients with a Gore-Tex™ tube inserted between the TCPC tunnel and the right atrium.

4.4 ECHOCARDIOGRAPHIC EXAMINATION

4.4.1 STUDIES I AND II (LONDON)

Standardized echocardiographic examination was performed under the same general anaesthetic as used in the MRI examination with a Philips iE33 ultrasound system (Philips Medical Systems, Andover, MA) using S8-3 or S5-1 probes as appropriate for patient size with frame rates of 80-120Hz. An apical 4-chamber view was recorded with the sector width adjusted to include the full endocardial border. For functional analysis, cardiac ultrasound data were transferred in a standard Digital Imaging and Communication in Medicine (DICOM) format to the functional analysis programs.

4.4.2 STUDIES III AND IV (HELSINKI)

Echocardiograms used for the study were performed as part of the treatment protocol at the Children's Hospital and were collected retrospectively from Xcelera (Philips Healthcare, Eindhoven, The Netherlands) database of stored echocardiographic images. Examinations included in this study had been performed prior to surgical stages 1, 2 and 3 and 0.5–2.7 years after stage 3 (Figure 5). These images had been recorded according to a standardized echocardiography protocol in our institute.

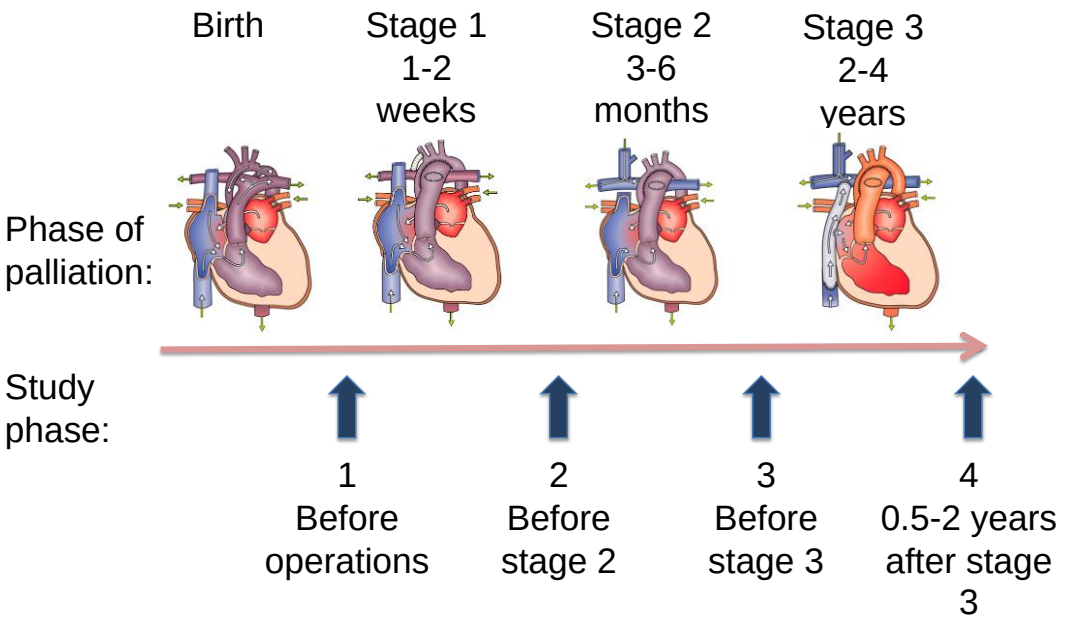

Figure 5. Operative treatment protocol and time points for clinical and echocardiographic examinations in the follow-up study (Study IV). Modifed from Sarkola 2017.

The severity of tricuspid and neo-aortic valve regurgitation was classified as haemodynamically significant (moderate or severe) or insignificant (none, trivial or mild), taking into account the vena contracta width and the area of the colour Doppler flow in relation to the valve annulus. The size of the LV was measured using the apical four chamber view and an auto left heart algorithm (Syngo USWP 3.0, Siemens Healthineers, Erlangen, Germany). The area of the LV in the apical four chamber view was traced and the volume of LV was calculated by the program algorithm using the assumption of an elliptical shape of the chamber.

4.5 RIGHT VENTRICULAR FUNCTIONAL ANALYSES

All functional analyses were performed using the apical four chamber view and the same loop was used for all analyses. All analyses were performed by the author who was blinded to the clinical presentation and outcome of the HLHS patients.

Manual measurement of FAC was done by drawing the RV areas with manual planimetry of the endocardial border at the end-diastole marked by the onset of the QRS complex and at end-systole when the RV area was the smallest (Figure 6). Tracing begun from the lateral side of the tricuspid valve annulus and ended at the medial side of the tricuspid valve annulus. Papillary muscles and trabeculations were left inside the tracing. FAC was calculated from the difference of these areas divided by the area in diastole and expressed as a percentage.

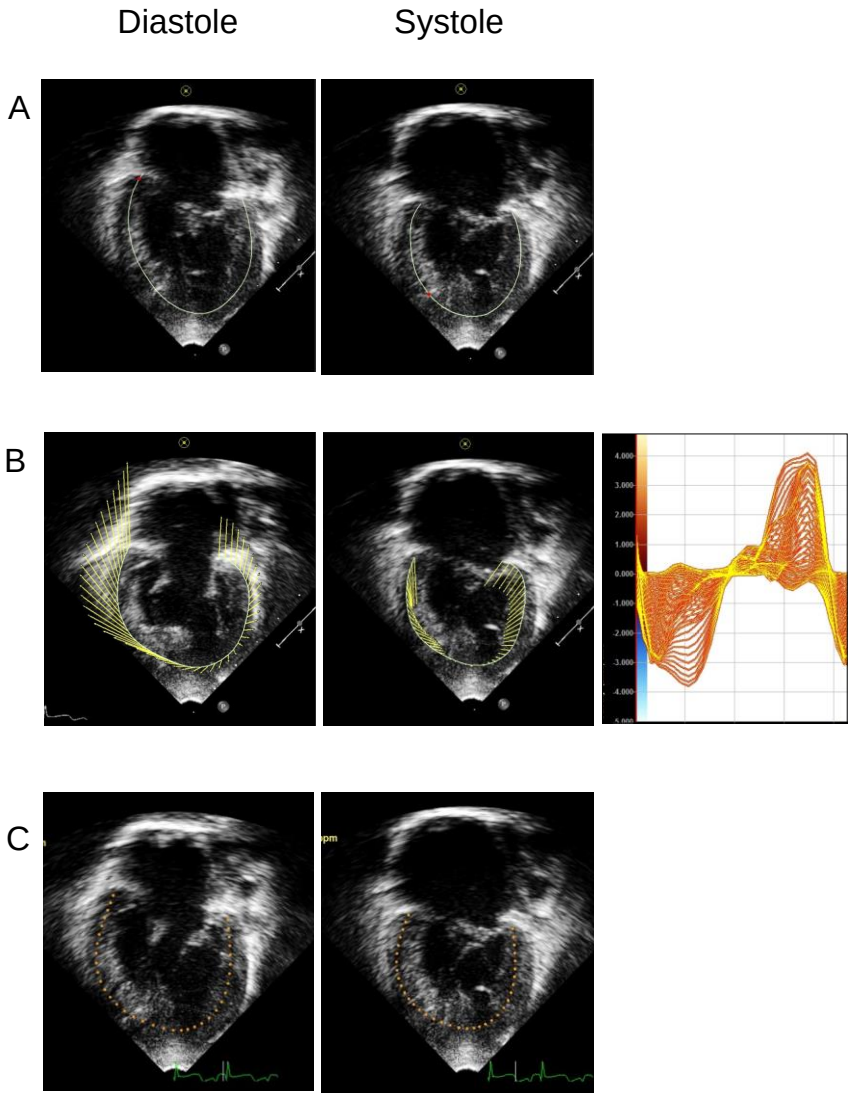

Figure 6. Examples of different echocardiographic based methods to assess myocardial function: a) manual tracing of FAC, b) VVI and c) QLAB.

4.5.1 VVI

Echocardiographic data for the functional analysis were transferred in a standard DICOM format to the VVI analyzing program (Syngo USWP 3.0, Siemens Healthineers, Erlangen, Germany). VVI program uses the effective frame rate of 30 Hz.

The apical four chamber view was used for the analysis (Figure 6). Manual tracing of the RV subendocardial surface was performed in a single still frame in mid-systole. Tracing began at the edge of the tricuspid valve annulus, extended to the apex of the ventricle without incorporation of the papillary muscle complex, and returned basally to the other edge of the tricuspid valve annulus. Velocity vectors were then automatically calculated

for each frame of the cardiac cycle by the VVI algorithm and displayed for the complete loop. The software divided the right ventricle automatically into six segments (three septal and three free wall segments) for regional and synchrony analysis. Tracings were accepted only when the subendocardial border was correctly followed throughout the whole cardiac cycle. When necessary, individual regions of the border were adjusted until the border was correctly tracked for each frame. Time to the peak V, S and SR values from the beginning of the QRS complex in all six segments was measured for synchrony analysis. The degree of mechanical dyssynchrony was quantified as the standard deviation of these values between six different cardiac segments divided by square root of the length of the cardiac cycle. The parameters calculated from the VVI analysis data were FAC, global and segmental myocardial longitudinal V, S, SR and mechanical DSI.

4.5.2 QLAB

The QLAB analysis program (Q-lab R 10.0, Philips Healthcare) uses native frame rates. In the QLAB analysis program points were placed at the end-diastole in the frame that was automatically chosen by the program, and these points were the edge of the tricuspid valve annulus on the RV free wall, ventricular septum and the apex (Figure 6). The rest of the tracing was then automatically carried out by the program. The points were adjusted until the myocardium was correctly followed throughout the whole cardiac cycle. The FAC was calculated by the QLAB program algorithm from the largest area in diastole to the smallest area in systole.

4.5.3 MRI (STUDIES I AND II)

MRI examinations were performed at Evelina London Children's Hospital under general anaesthesia. MRI scans were performed with a Philips 1.5-T Achieva scanner (Philips Medical Systems, Best, The Netherlands) and re-evaluated using ViewForum EWS version 2.0 (Philips Medical Systems, Best, The Netherlands). 2D steady-state free precession cine imaging oriented to the short axis of the RV was used to calculate the ventricular volumes using the disk summation method. End-diastolic and end-systolic contours were manually traced (excluding major trabeculations from the volume) to determine EDV, ESV, and EF values (Figure 7).

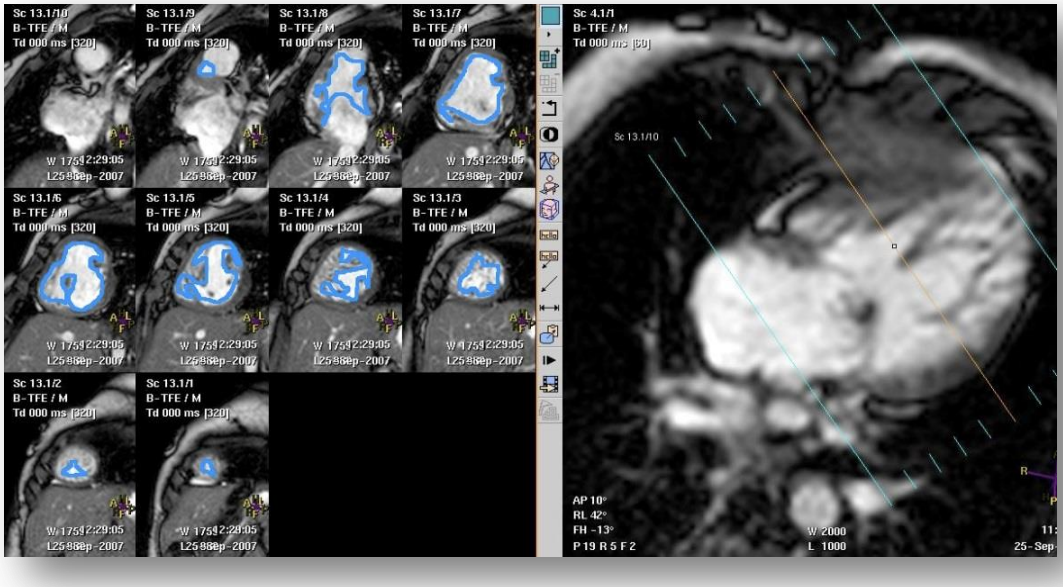

Figure 7. Measurement of EF by MRI from a stack of contiguous slices aligned in the short-axis plane of the tricuspid valve.

4.6 CARDIAC CATHETERIZATION (STUDY IV)

Cardiac catheterization with oximetry, pressure measurements and angiography was performed prior to stage 2 operation and before and after stage 3 operation. The procedure was performed under general endotracheal anaesthesia. Data collected from cardiac catheterizations included pressure measurements in the RV, ascending aorta, descending aorta and pulmonary arteries in addition to angiographic measurements of PAs and the aorta.

4.7 STATISTICAL METHODS

Data are expressed as mean ± SD or median (range). The Kolmogorov-Smirnov test with Lilliefor's significance correction was used to test the normality of the parameters. Logarithmic transformation was used to normalize skewed distributions for the t-tests, multiple regression analysis and RM ANOVA models. Clinical demographics and global cardiac functional data for the study groups were compared using t-tests or the Mann-Whitney U-test for continuous parameters. The Cochran-Mantel-Haenszel test for non-continuous parameters and Fisher's exact test or Pearson Chi-squared test for frequencies. Bonferroni corrected t-tests were used for repeated analyses. Multiple regression analysis was used to find factors affecting myocardial function. Correlations (r) were calculated using the Pearson's correlation coefficient for normally distributed data and the Spearman's rank correlation coefficient for abnormally distributed data. Stepwise linear regression analysis was performed to determine whether any of the VVI

parameters predicted MRI derived EF better than others. Results were reported as ß-coefficients and 95% CIs.

Intraobserver and interobserver reproducibility were assessed for 10 randomly selected patients using mean differences and from the corresponding repeated measures using intraclass correlation coefficients (ICC) with 95% CIs. The times required for different echocardiographic modalities were measured for all analyses. Time started when echocardiography loop was ready for analysis at analyzing program and ended when measurements were accepted and ready for data saving.

P-values of <0.5 were used to define statistical significance. SPSS for Windows version 22 (SPSS Inc/IBM Corporation, Somers, NY, USA) was used to perform statistical analyses.

5 RESULTS

5.1 COMPARISON OF METHODS USED FOR EVALUATION OF MYOCARDIAL FUNCTION (I, II)

Manual FAC was measured for all 51 patients, VVI-analysis was possible for 49 patients (96%) and QLAB-analysis for 44 patients (86%). The VVI-analysis was not possible in two cases due to difficulties in tracking. The QLAB-analysis was not possible in seven cases because the tracking was not adequate through the whole cardiac cycle. Four of these patients had a globular left ventricle and three had either a slit-like or no discernable left ventricle. The duration of FAC analysis, including manual corrections, was shortest for the manual method 1 min 39 sec (55 sec–2 min 20 sec) for VVI 4 min 5 sec (3 min 9 sec–5 min 30 sec) and for QLAB 2 min 58 sec (1 min 40 sec–2 min 44 sec) ($p<0.001$).

The intraobserver and interobserver ICC data are shown in Figure 8. Intraobserver and interobserver reproducibility was excellent or good for all automated parameters: excellent for VVI-parameters (ICC>0.9) and good for QLAB (ICC>0.7). For manually derived FAC, the interobserver reproducilbility was poor, particularly in regard to the end systolic area (Figure 8). Bland-Altman analysis of the mean intraobserver and interobserver differences with limits of agreement of different methods and parameters are presented in Table 7.

Table 7. Intraobserver and interobserver differences and limits of agreement of different methods by Bland-Altman analysis.

Method and parameter	Intraobserver difference and limit of agreement	Interobserver difference and limit of agreement
VVI-FAC	0.04±1.2%	-1.9±3.4%
S	0.7±1.7%	1.4±3.1%
SR	-0.003±0.1 1/s	-0.1±0.4 1/s
V	-0.06±0.3cm/s	00.5±0.5cm/s
QLAB-FAC	1.0±1.6%	1.5±2.9%
Manual FAC	2.0±8.0%	7.411.7%.

All functional measurements except for myocardial systolic V correlated significantly with MRI derived EF (R>0.4) (Figure 9). For FAC measurements, higher r was detected using automated software packages (VVI-FAC r=0.7 and QLAB-FAC r=0.6) than with the manual method (r=0.4). The correlation coefficients between VVI and the manual method were statistically different ($p=0.030$), but there was no difference between the two automated techniques ($p=0.500$). The highest correlation for the VVI measurements was detected against FAC-VVI (Figure 9.), and in stepwise regression analysis, including all VVI functional measurements, FAC-VVI was the best predictor of MRI derived EF (intercept ß-coefficient 27.8 (18.9–36.8); FAC-VVI, ß-coefficient 0.9 (0.7–1.2), $p<0.001$; S, $p=0.800$; SR,

p=0.700; V, p=0.060).

Stage of palliation or morphology group did not have an impact on the r of MRI measurements with any echocardiographic technique (stage: manual p>0.700, VVI p>0.600, QLAB p>0.300; morphology: manual p>0.4, VVI p>0.2), except QLAB FAC correlation with MRI EF was lower for slit/none LV morphology group (r=0.2) than globular morphology group (r=0.8, p=0.020).

The Bland-Altman assessment of the agreement between the different techniques to measure FAC is shown in Figure 10. Both automated techniques tended to produce lower FAC values compared with manual measurements (VVI-FAC -7.2%, p<0.001 and QLAB-FAC -9.8%, p<0.001). VVI-technique, too, produced 2.4% units higher values than QLAB-technique (p<0.0001). Limits of agreement were wide between manual and automated techniques but narrower between the two automated techniques (Figure 10).

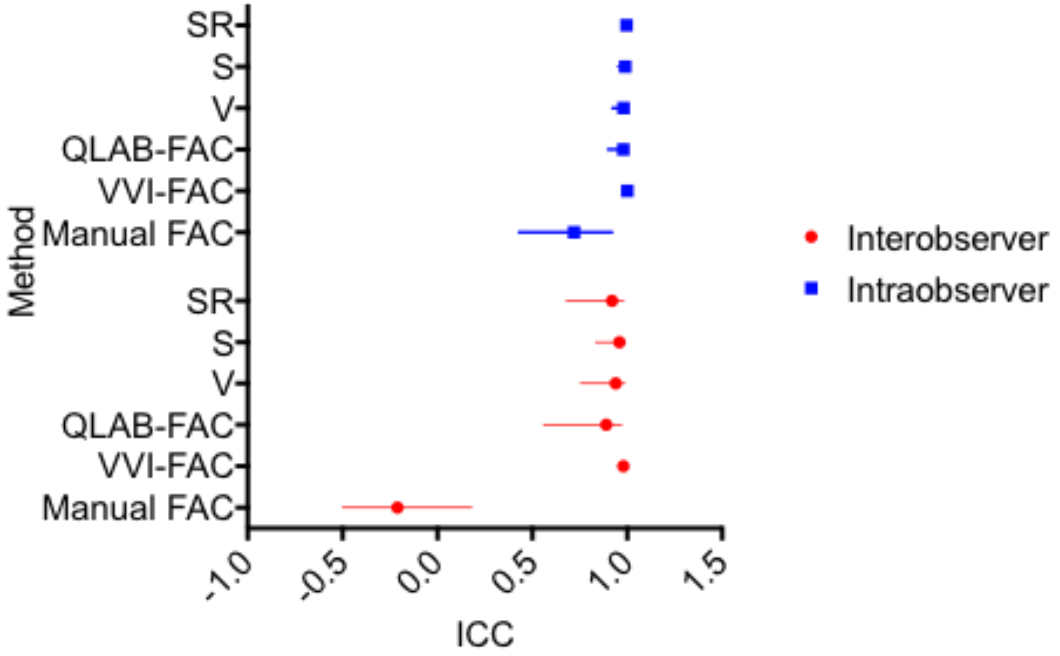

Figure 8. Intraobsever and interobserver reproduciblity (measured by ICC) for different methods.

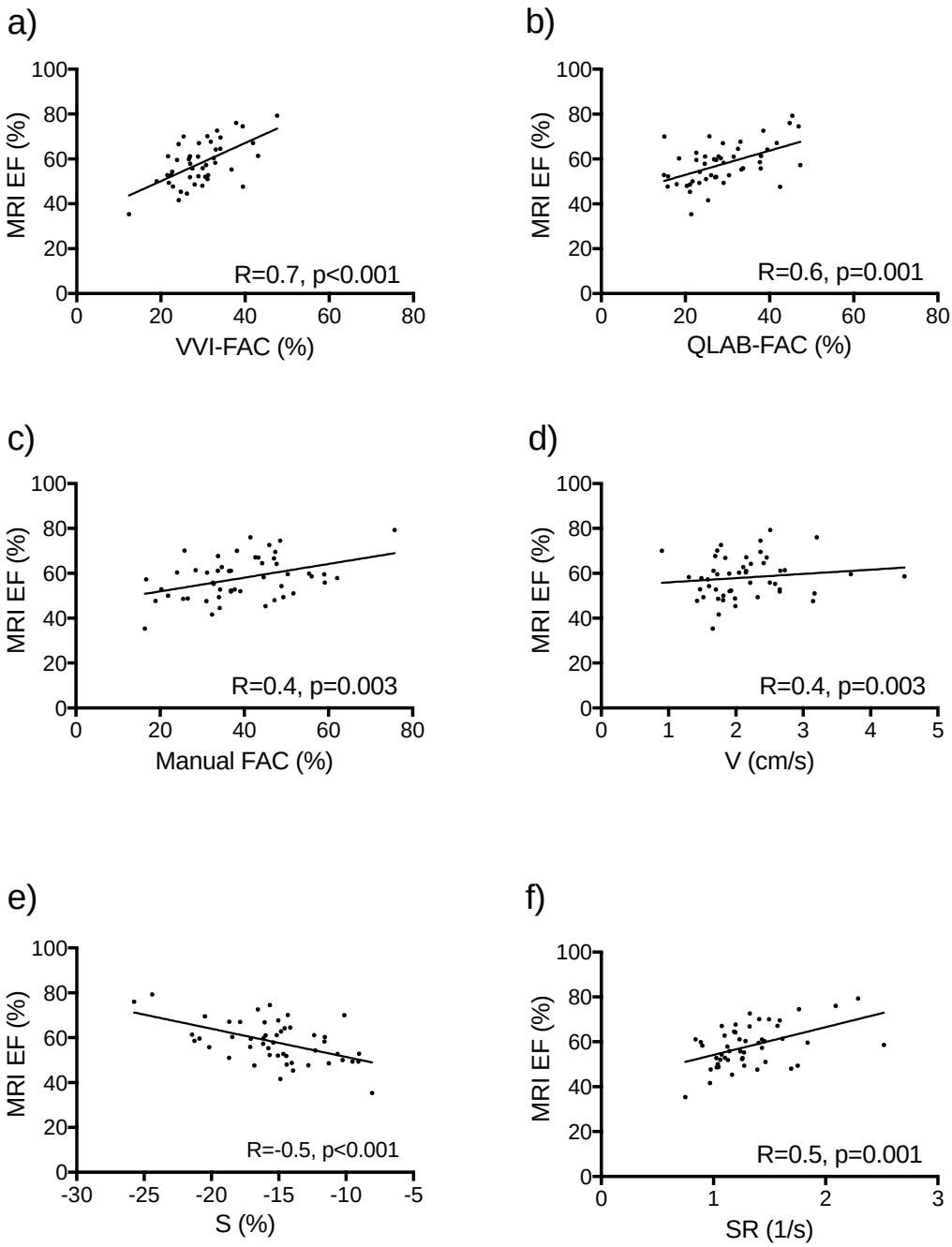

Figure 9. Correlations between MRI EF and echocardiography based methods of measuring systolic function in HLHS.

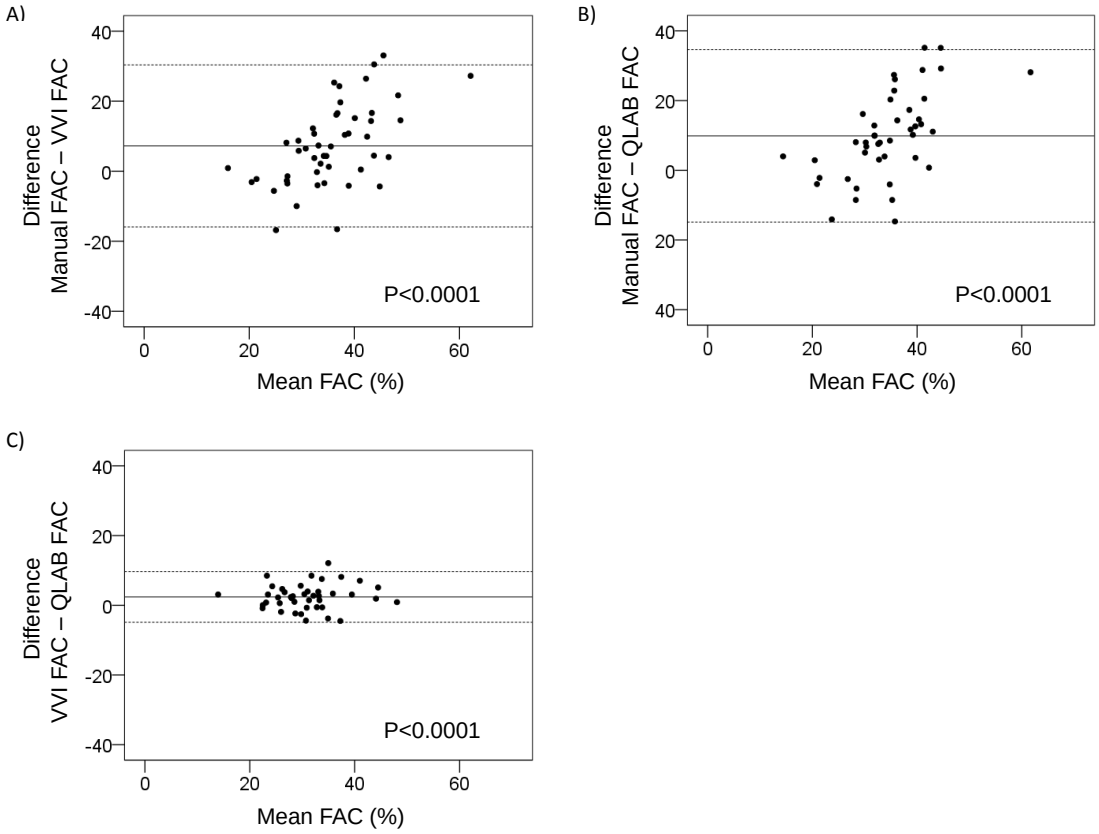

Figure 10. Bland-Altman analysis comparing different techniques to measure FAC in HLHS patients.

5.2 CLINICAL STUDIES (III AND IV)

5.2.1 THE IMPACT OF PRENATAL DIAGNOSIS ON MYOCARDIAL FUNCTION (III)

Twenty-five infants (38%) had had a prenatal diagnosis and 41 (62%) were diagnosed postnatally (Figure 11). The prevalence of a prenatal diagnosis of HLHS increased during the study era (from 22% in the first year to 75% in the last year) and prenatal diagnosis was done at a median gestational age of 22 (17–27) weeks. All infants in our study cohort who had prenatal diagnosis were delivered at the University Hospital of Helsinki. Infants without prenatal diagnoses were born in delivery hospitals around Finland and were transported to Helsinki University Hospital after diagnosis and stabilization. The mean transportation distance from delivery hospital to operative centre was 225 ± 202 km.

For postnatally diagnosed infants, the mean time lag from delivery to diagnosis was 1.9±1.8 days (range 0–7 days). Oxygen saturation screening for the detection of cardiac defects was started after 2008 in most delivery hospitals in Finland. Early oxygen saturation screening was performed for

eight postnatally diagnosed infants. Postductal saturation in oxygen saturation screening was initially normal (>95%) for two infants with a postnatal diagnosis of HLHS, and in both of them, saturation became abnormal (<95%) before discharge. The majority, 33 out of 41 (80%) of postnatally diagnosed neonates did not have oxygen saturation screening. Five of these neonates had delayed diagnosis more than 72 hours after birth. There was no delayed diagnosis among the neonates that had oxygen saturation screening.

Less preoperative acidosis and lower peak concentrations of creatinine and alanine aminotransferase suggested less end-organ dysfunction was observed in the prenatally diagnosed neonates compared to postnatally diagnosed neonates with HLHS (Table 8). No differences in the highest recorded lactate concentrations were detected between the study groups, although lactate values were found to be above the normal range in both groups.

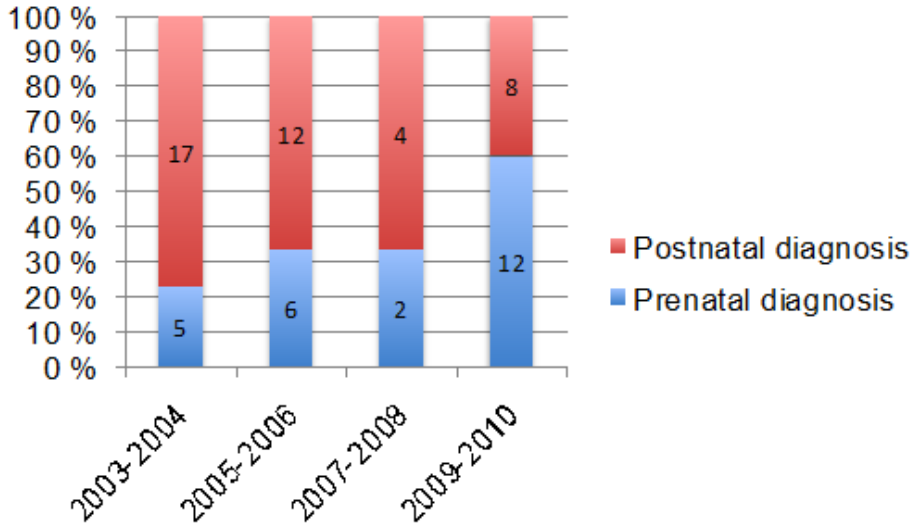

Figure 11. Change in the incidence of prenatal and postnatal diagnosis of HLHS in Finland between 2003–2010 in two-year intervals.

Table 8. Clinical data of prenatally and postnatally diagnosed HLHS neonates.

	Prenatal diagnosis (n=25)	Postnatal diagnosis (n=41)	p
Birth data			
Maternal age (years)	29 (22–36)	27 (20–40)	0.700
Gestational weeks at birth(weeks)	39 (37–41)	40 (36–42)	0.800
Male (%)	13 (52%)	25 (61%)	0.500*
Birth weight (grams)	3440 (2690–4300)	3520 (2220–4572)	0.700
Apgar score (points)	9 (7–9)	9 (6–10)	0.700†
Laboratory values			
Lowest arterial pH	7.30±0.04	7.25±0.09	0.005
Highest lactate (mmol/l)	3.5±1.9	3.8±3.9	0.100
Highest creatinine (μmol/l)	78±18	81±44	0.050
Highest alanine aminotransferase (U/l)	33±38	139±174	<0.001
Stage I operative management			
BT shunt (%)	7 (28%)	16 (49%)	0.400*
RV-PA shunt (%)	18 (72%)	34 (51%)	0.400*
Operative age (days)	5.7±1.8	7.5±2.9	0.008
<30d mortality n (%)	0 (0%)	4 (10%)	0.150*

5.2.2 FOLLOW-UP DURING THE OPERATIVE PROTOCOL (IV)

Twenty-three children were palliated with the BT shunt and 40 with the RV-PA conduit over the study period. A flow chart of patient outcomes through the surgical stages is presented in Figure 12. There were no differences in the frequency of prenatal diagnosis, HLHS anatomic subtype, gestational age, preoperative haemodynamics or birth weight between study groups (Table 9).

Table 9. Demographics and operative data of 63 HLHS patients divided in two groups according to the type of shunt used in stage I operation.

	BT n=23 mean±SD / median (range) / n (%)	RV-PA n=40 mean±SD / median (range) / n (%)	P
Before operations	n=23	n=40	
Male / female (n(%))	13 (56.5)/10(43.5)	23(57.5)/17(42.5)	1.0
Gestational age at birth (weeks+days)	39+3 (36+0–41+6)	39+3 (36+0–42+0)	0.8
Birth weight (kg)	3.5±0.4	3.5±0.4	0.9
Prenatal diagnosis (n (%))	7 (30.4)	17 (42.5)	0.3
HLHS anatomic subtype MS/AS ; MS/AA ; MA/AA (n(%))	9(39.1)/6(26.1)/8(34.8)	17(42.5)/9(22.5)/14(35.0)	0.9
Lactate (mEq/l)	1.0±0.2	1.0±0.2	0.8
Stage I palliation	n=23	n=40	
Age (days)	7.2±2.6	6.6±2.7	0.4
Perfusion time (minutes)	146.0±47.4	177.6±37.1	0.006
Aortic cross clamp time (minutes)	43.5±25.2	63.1±25.3	0.007
Length of ventilation (days)	6.0 (3-85)	6.5 (2-21)	0.3
Length of ICU stay (days)	11.0 (6-94)	12.0 (4-26)	0.3
Length of hospital stay (days)	27.5 (14-157)	27.0 (12-142)	0.5
Stage II palliation	n=17	n=35	
Age (months)	4.6±2.5	6.2±2.0	0.02
Weight (kg)	5.7±1.1	6.8±1.1	0.005
Perfusion time (minutes)	101.3±74.8	87.5±49.6	0.5
Aortic cross clamp time (minutes)	14.1±30.6	9.6±27.9	0.7
Additional procedures (n (%))	9 (52.9%)	16 (45.7%)	0.6
Length of ventilation (days)	1 (1-11)	1 (1-3)	0.01
Length of ICU stay (days)	3 (2-31)	3 (1-7)	0.02
Length of hospital stay (days)	19 (8-235)	14 (7-84)	0.08
Stage III palliation	n=17	n=30	
Age (months)	39.0±5.3	37.8±7.2	0.6
Weight (kg)	13.9±1.5	14.6±2.0	0.1
Perfusion time (minutes)	123.6±61.3	91.1±26.4	0.02
Aortic cross clamp time (minutes)	36.5±29.4	34.2±23.7	0.8
Additional procedures (n (%))	10 (58.8)	16 (48.5)	0.4
Length of ventilation (days)	1.5±1.6	1.2±0.7	0.3
Length of ICU stay (days)	5.7±3.7	4.3±2.4	0.1
Length of hospital stay (days)	28.5 (13-150)	17.0 (10-69)	0.06

Post-Stage III evaluation	n=17	n=30	
Time from stage III (months)	19.0±8.4	19.1±5.6	1.0
Age (months)	56.1±9.1	57.4±9.3	0.7

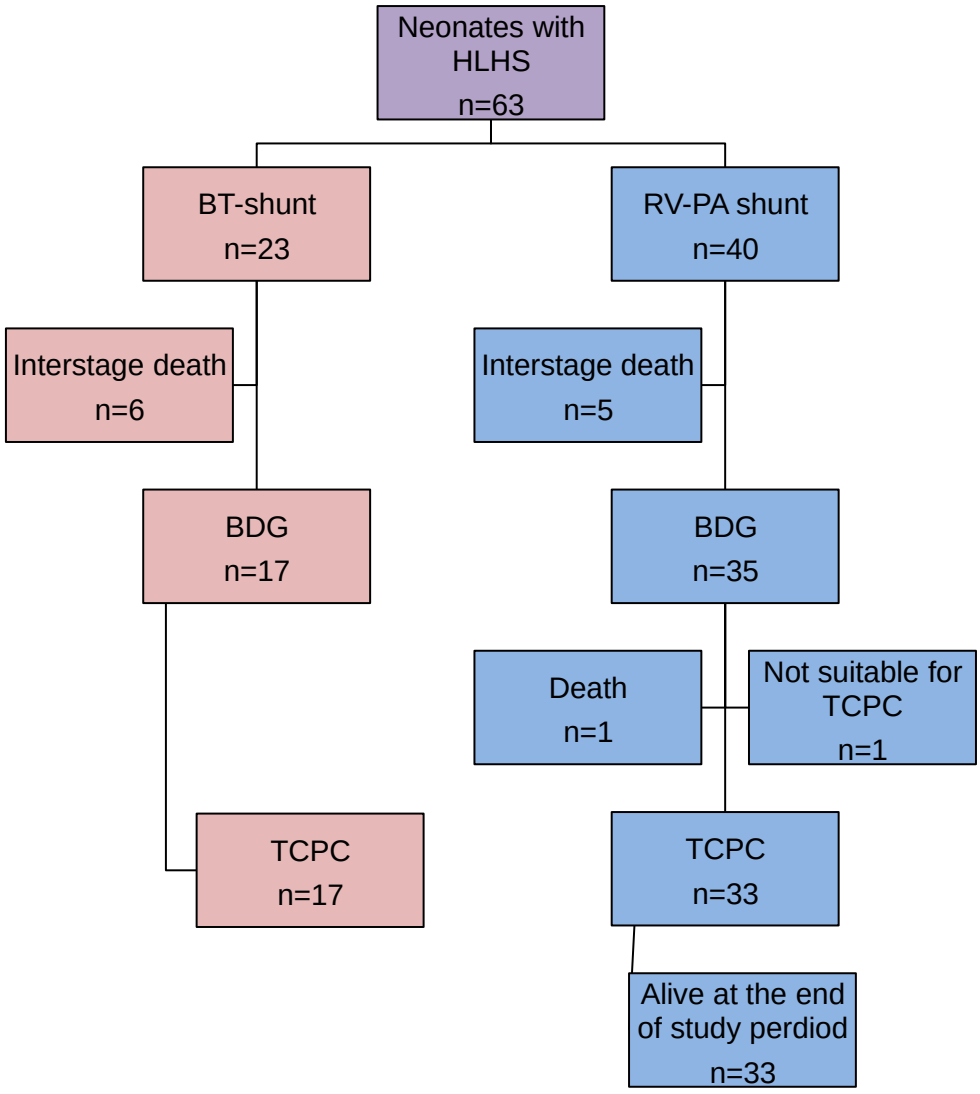

Figure 12. Flow chart of patient outcomes during treatment protocol for HLHS.

At stage 1 operation, cardiopulmonary bypass time and aortic cross clamp time were longer in the RV-PA group. A 6mm conduit was used in all patients in the RV-PA group. Shunt size in the BT group was 3.5 mm in nine (39%) and 4.0 mm in 14 (61%) patients. There were no differences in the duration of ventilation, the length of the intensive care unit (ICU) stay or length of hospital stay between groups (Table 9).

Stage 2 palliation was performed earlier in the BT group largely due to clinical deterioration in these patients. Clinical deterioration included severe desaturation or pulmonary overcirculation and heart failure. At the time of stage 2 operation, patients with BT shunts had lower weight, but no difference in weight centile (data not shown) was observed. Both systolic and diastolic blood pressures were lower in patients with a BT shunt at the time of the stage 2 operation (81.4±12.4/43.4±10.2 mmHg vs. 93.7±11.6/53.5±8.8 mmHg, p=0.005, p=0.003). During the stage 2 operation, cardiopulmonary bypass was used for all patients in both groups. No differences in cardiopulmonary bypass or aortic cross clamp times were observed between the groups. The BT group patients had slower postoperative recovery. There were no other differences in the clinical data or operative factors during stage 2 (Table 9).

At stage 3 operation, there were no differences between groups in clinical data. Cardiopulmonary bypass time was longer in the BT group (Table 9). A fenestration was created in all patients. Postoperatively, mean duration of pleural and/or visceral effusions were longer for the BT group (30±15 days vs. 15±12 days, p=0.030), and length of hospital stay tended to be longer for the BT group but this did not reach statistical significance (34±32 vs. 21±12 days, p=0.060). Spontaneous closure of the Fontan fenestration was observed in 44% of the patients during follow-up with no difference between groups.

Cumulative total cardiopulmonary bypass time during the whole study period was 286±146 min for the BT group and 323±121 min for the RV-PA group (p=0.300). Recoarctation incidence was 24% during the study period, 10 patients were operated on and six patients underwent balloon angioplasty with no difference between the groups (p=0.900). There was no difference on additional procedures between shunt types (Table 10).

Table 10. Additional procedures performed on the HLHS patients during the study period.

	BT group	RV-PA group	p
Tricuspid valvuloplasty	7 (30%)	6 (15%)	0.100
Pulmonary artery repair	9 (39%)	24 (58%)	0.500
Aortic arch repair	4 (17%)	10 (24%)	0.900
Patients with additional procedures (n (%))	15 (65%)	28 (68%)	0.600
Total number of procedures	27	53	0.600

5.2.3 MYOCARDIAL FUNCTION IN HLHS (III AND IV)

Myocardial function before operations: impact of prenatal diagnosis (III)

Global RV function before stage I operation was better in the prenatally diagnosed patients compared with the postnatally diagnosed HLHS patients (FAC: 27.9±7.4 vs. 21.1±6.3%, p<0.001; V: diastole 1.6±0.6 and systole 2.0±1.1 cm/s vs. diastole 1.3±0.4 and systole1.4±0.4 cm/s, p=0.004, p<0.001; S: 8.5±4.9 vs. 5.8±2.5%, p=0.008; SR: 1.1±0.6, 1.3±1.0 1/s vs. 0.7±0.2, 0.7±0.3 1/s, p=0.004, p=0.003). In segmental analysis, patients with a prenatal diagnosis had better myocardial function for all six segments, as measured by the V, S and SR both in systole and in diastole (Figure 13). Neonates with early postnatal diagnosis (<3 days) tended to have better myocardial function than those with delayed postnatal diagnosis (>3 days) (Figure 14). No difference was detected between the study groups in mechanical synchrony measurements as expressed by the means and standard deviations of the time to peak strain rate (27.4±15.3 ms vs. 32.5±17.3 ms, p=0.300) or to peak myocardial velocity (28.6±24.4 ms vs. 31.1±23.2 ms, p=0.700). There was no correlation between mechanical synchrony and myocardial function.

The incidence of tricuspid valve regurgitation was similar in both study groups and its severity was found not to be related to myocardial function or mechanical synchrony. There was a weak correlation between the left ventricular size and FAC (r=-0.3, p=0.014). The size of the left ventricle and the diameter of the ascending aorta did not correlate with global or segmental myocardial V, S or SR measurements (r<0.2, p>0.500).

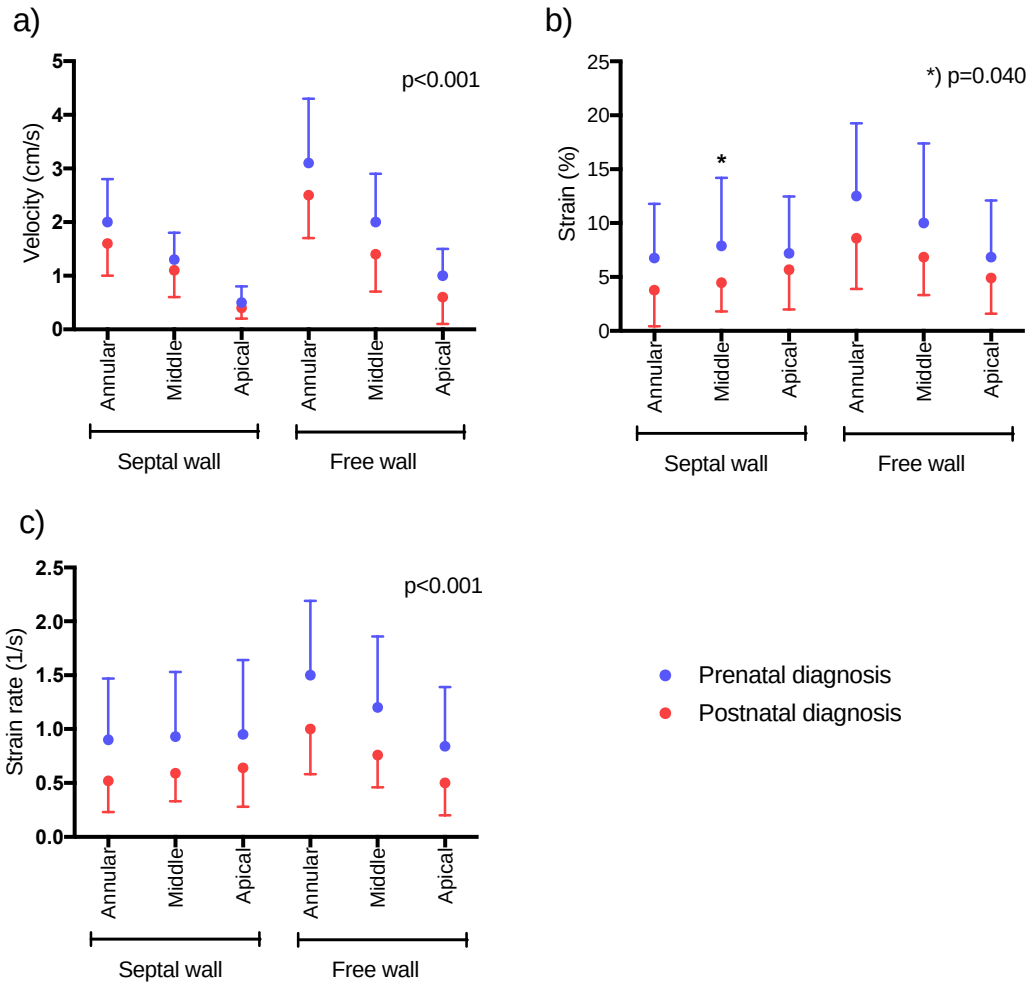

Figure 13. The impact of prenatal or postnatal diagnosis of HLHS neonates on segmental myocardial function before stage I operation measured by V, S or SR.

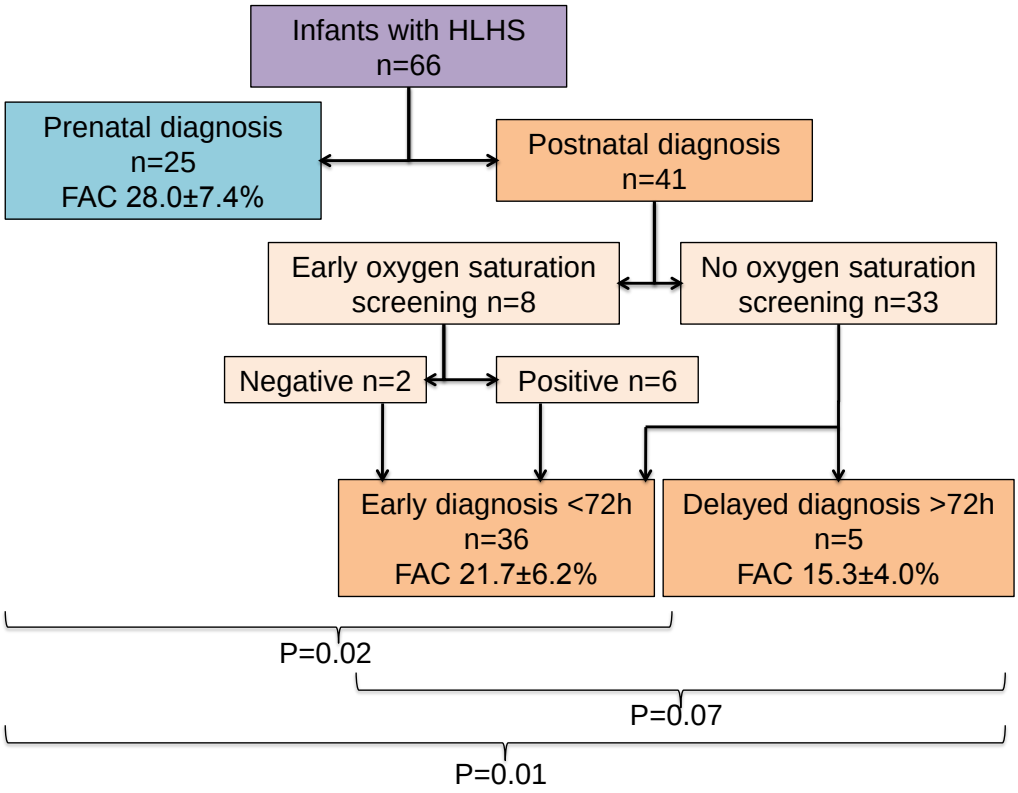

Figure 14. The impact of timing of HLHS diagnosis on myocardial function measured by FAC.

Myocardial function during staged treatment protocol (IV)

No differences in myocardial function were detected between groups ($p>0.100$) at baseline before stage 1 palliation commenced (Figure 15). Subsequently there was growth of RV in the BT group after stage 1 operation, and before stage 2 operation, and the RV size was larger and systolic function measured by FAC worse in the BT group than in the RV-PA group. However, no difference between shunt types was observed or in any other measurements of myocardial function (Figure 15). Poor myocardial function (FAC<16%, <-2 SD in study population) was detected in five patients in both groups. In the RV-PA group, SR decreased during the interstage period of 1–2 (SR -0.2±0.5 1/s, $p=0.020$) (Figure 15).

The RV size decreased and myocardial function measured by S or SR recovered and, in the BT-group in both groups after the stage 2 operation also FAC increased (FAC +8.9±9.4%, $p=0.001$) (Figure 15). Before stage 3 operation, no difference in myocardial function or RV size was seen between shunt types.

After stage 3 operation, S diminished in the RV-PA group (Figure 15). Myocardial function as measured by FAC, S and SR was better in the BT group after stage 3 completion as compared to the RV-PA group (Figure 15).

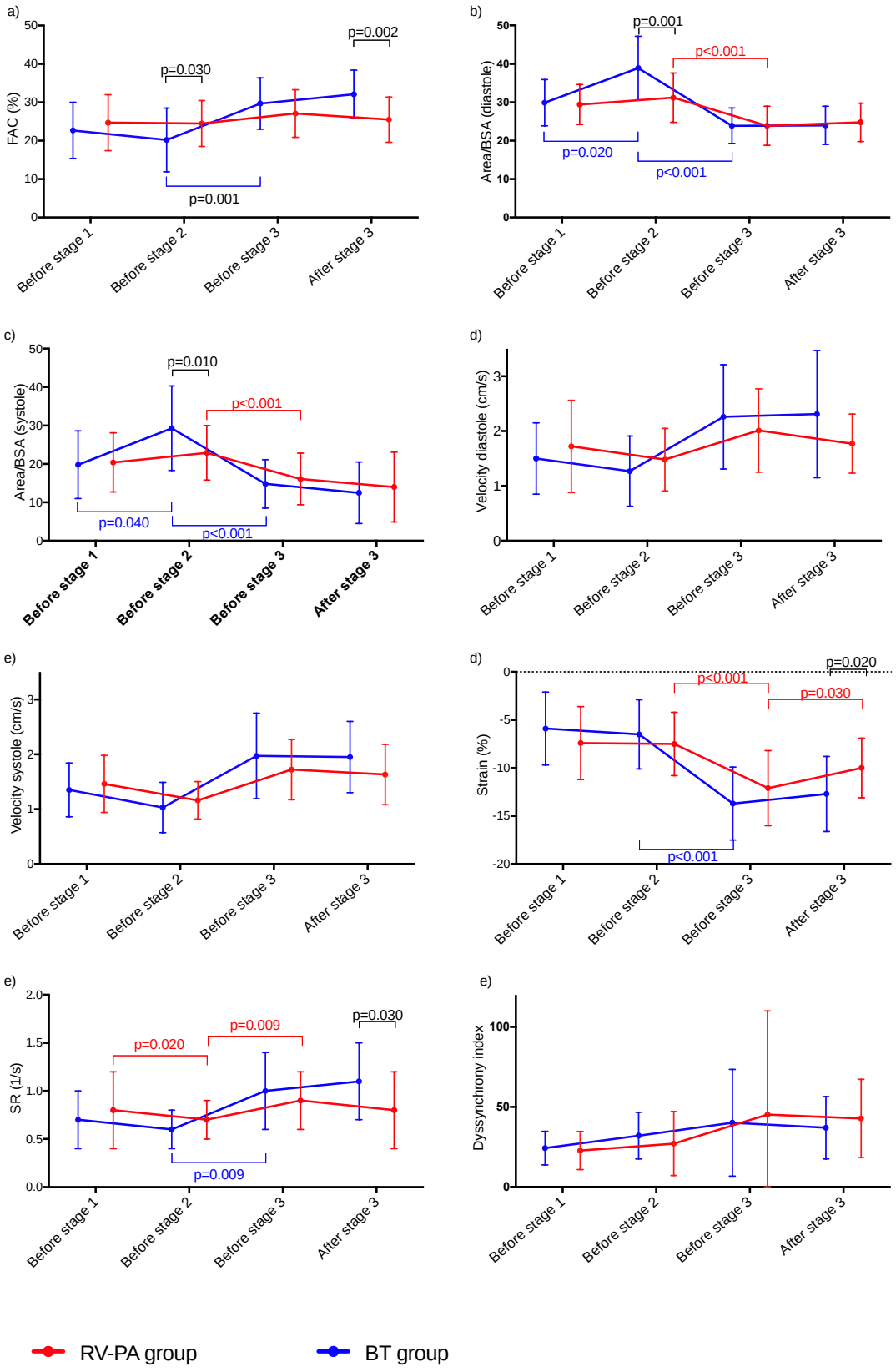

Figure 15. Myocardial function measured by a) FAC, b) area in diastole, c) area in systole, d) diastolic velocity, e) systolic velocity, f) strain, g) strain rate and h) dyssynchrony index during treatment protocol of HLHS.

There was no difference between study groups in regional V, S or SR measurements at any stage (Figure 16). Mechanical dyssynchrony increased with time in both groups but there was no difference between shunt types at any time point (p>0.400) (Figure 15). No correlation was detected between myocardial dyssynchrony and myocardial function (r<0.500, p>0.300) at any stage.

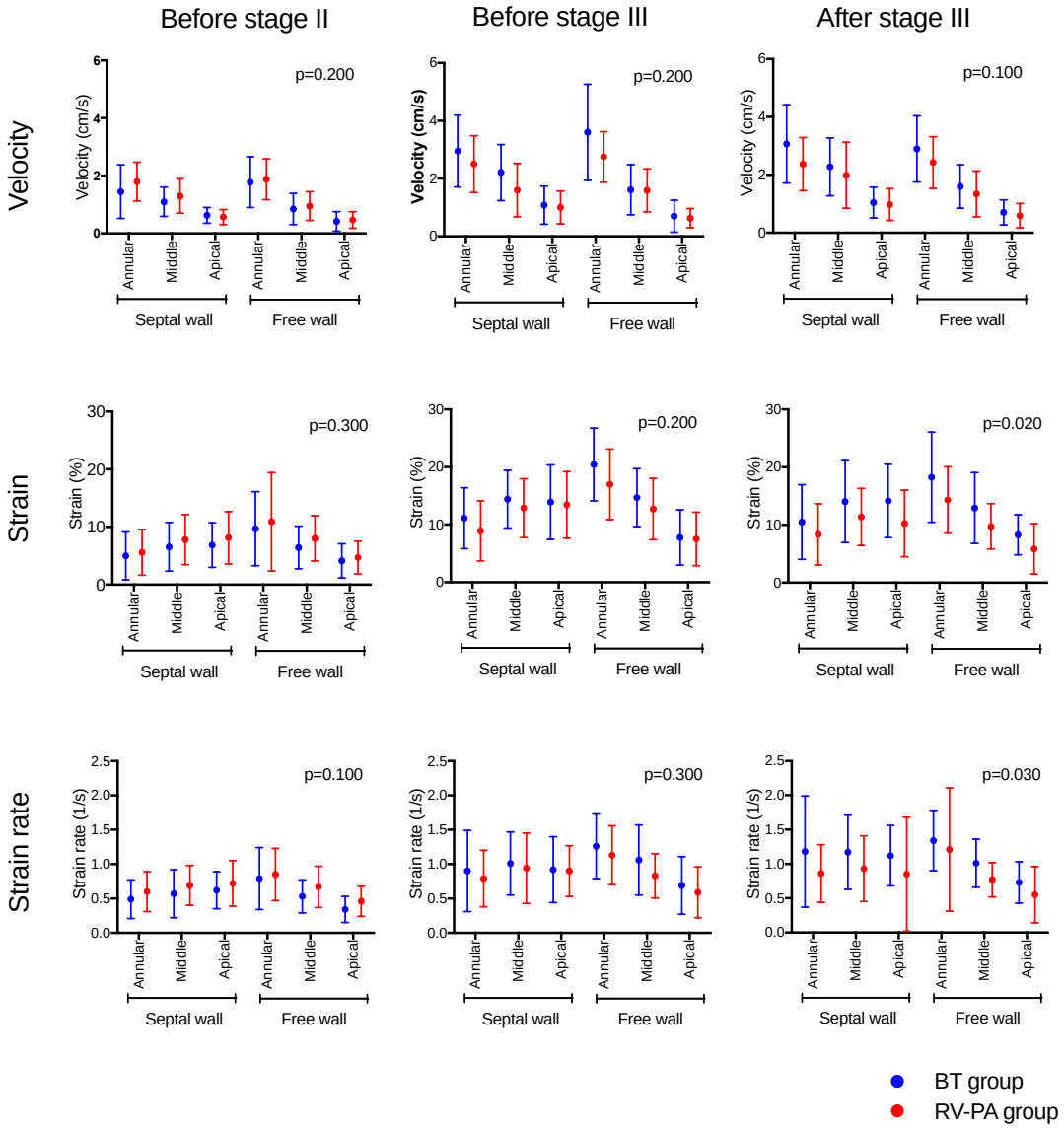

Figure 16. Regional myocardial function, measured by V, S and SR, according initial shunt type during treatment protocol of HLHS.

The incidence of significant tricuspid valve regurgitation at baseline was similar in both groups. None of patients had severe tricuspid insufficiency before the Norwood operation. After stage 1, significant tricuspid valve regurgitation was more common in the BT group but thereafter, there was no difference in the incidence of significant tricuspid valve regurgitation (Table 11). During the follow-up, tricuspid valvuloplasty was performed in seven

patients in the BT shunt group and six patients in the RV-PA conduit group (p=0.100).

Table 11. Incidence of significant tricuspid valve regurgitation and tricuspid valve plasties performed during treatment protocol.

	BT group		RV-PA group		
	Tricuspid regurgitation (n (%))	Tricuspid Valvuloplasty (n)	Tricuspid regurgitation n (%)	Tricuspid Valvuloplasty (n)	P
Before stage 1	3 (13%)		9 (25%)		0.400
Before stage 2	8 (44%)	1	4 (11%)		0.010
Before stage 3	6 (33%)	6	10 (29%)	6	0.800
After stage 3	3 (17%)		8 (25%)	1	0.600

Shunt type and stage of palliation were the only characteristics remaining in the final multiple stepwise regression analysis model. Blood pressures, oxygen saturations or a fenestration were not associated with myocardial function at any stage (p>0.050). In the final model, stage of palliation and initial shunt type had an association with myocardial function measured by FAC (regression coefficient ß1 41.4 (95% CI, 27.8–55.3), p=0.004). The direction of the association between shunt type and myocardial function changed during the treatment protocol: between stages 1 and 2, FAC was higher in patients with an RV-PA conduit and thereafter, FAC was higher in patients with a BT shunt.

5.2.4 MORTALITY

The incidence of prenatal diagnosis did not have a significant impact on early mortality. Although there were no early deaths among the prenatally diagnosed neonates and infants early deaths occurred in four neonates that had been diagnosed postnatally (10%; p=0.150). One postnatally diagnosed neonate died preoperatively due to severe myocardial dysfunction and haemodynamic compromise.

Shunt type during stage 1 operation did not have an impact on 30-day mortality (BT group n=2 (8.7%) and RV-PA group n=1 (2.5%), p=0.500). One neonate died during operation and two infants died postoperatively suddenly with no obvious explanation in autopsy. Interstage mortality, including 30-day mortality, between stages 1 and 2 was 26.1% (six patients) in the BT group and 12.5% (five patients) in the RV-PA group (p=0.2). Four patients died

after stage 2 evaluation, three before stage 2 (two of the BT group and one of the RV-PA group) and one postoperatively after stage 2 (of the RV-PA group). These non-survivors had lower myocardial function before stage 2 palliation as compared to those who survived until stage 3 palliation (n=51) (FAC 15.7±6.0% vs. 23.7±6.8%, p=0.030). One patient in the RV-PA group died before stage 3 operation. There was no mortality after stage 2 operation for the BT group. Overall mortality during the study period was 19.0%. All mortality occurred either in the interstage period between stages 1 and 2 or between stages 2 and stage 3. None of the patients underwent cardiac transplantation during the study period.

5.2.5 CARDIAC CATHETERIZATIONS

Cardiac catheterization was performed in 16 (70.0%) of patients of the BT group and 36 (90%) in the RV-PA group before stage 2 palliation (p=0.600) compared with all patients before stage 3 operation (p=1.000). Ten patients (58.8%) in the BT group and 23 (70.0%) in the RV-PA group (p=0.800) in median two years after stage 3. Before stage 2 operation higher end-diastolic RV pressure (7.7±2.4 mmHg vs. 6.5±1.5 mmHg, p=0.040) and lower diastolic pressure in the aortic arch (34.8±5.9 mmHg vs. 40.6±9.1 mmHg, p=0.030) were observed in the BT group compared to RV-PA group. There were no differences between groups in arterial saturations, size of pulmonary arteries or the McGoon index at any stage (Table 12).

Table 12. Catheterization data of study groups: a Blalock–Taussig (BT) shunt versus a right ventricle to pulmonary artery conduit (RV-PA) over the study period. CIx = Cardiac index, LPA Left pulmonary artery, RPA = Right pulmonary artery, RV EDP = Right ventricular end-diastolic pressure.

	Before stage 1		**Before stage 2**		**Before stage 3**		**After stage 3**	
	BT / RV-PA	P	BT / RV-PA	P	BT / RV-PA	P	BT / RV-PA	P
CIx	NA		NA		4.7±2.1 4.7±3.8	1.0	3.5±1.4 3.3±0.8	0.7
Mc-Goon index	NA		1.6±0.3 1.8±0.5	0.2	1.6±0.5 1.7±0.4	0.5	1.0±0.5 1.6±0.6	0.4
LPA	5.5±1.0 5.1±1.0	0.09	5.6±1.5 5.3±1.5	0.4	7.3±1.3 7.4±1.6	0.9	8.5±2.6 8.8±2.2	0.7
RPA	5.4±0.7 5.2±0.8	0.6	6.5±1.9 5.9±1.6	0.2	8.2±1.5 8.6±1.9	0.4	9.4±2.5 9.4±1.8	1.0
RV EDP	NA		7.7±2.4 6.5±1.5	0.001	7.2±2.0 8.5±1.0	0.6	6.8±1.4 7.0±1.7	0.8

6 DISCUSSION

Myocardial dysfunction is an important risk factor for operative and interstage mortality in HLHS patients during the treatment protocol. Myocardial dysfunction is related to failure of the Fontan circulation and the need for transplantation after Fontan completion. Poor cardiac function is associated with complications such as arrhythmias, protein losing - enteropathy and tricuspidal regurgitation (Altmann 2000, Chetan 2013, Friedman 2011, Ghanayem 2012, Hughes 2011, Jean-St-Michel 2016, Kotani 2009, Photiadis 2012, Simsic 2005, Tweddell 2012, Walsh 2009). Preserved cardiac function is essential for favourable prognosis, therefore the correct assessment of myocardial function with validated methods in clinical follow-up has great value for HLHS patients. The aetiology of myocardial dysfunction is poorly understood and therefore, the factors affecting cardiac function throughout the treatment protocol should be further investigated.

6.1 METHODOLOGICAL CONSIDERATIONS

In clinical practice, cardiac functional assessment in HLHS patients has largely been based on subjective, so called “eye ball method”, because it is quick and easy. However, previous studies have shown that subjective evaluation is poor (Bellsham-Revell 2013a, Muthurangu 2005) and therefore, quantitative methods of cardiac assessment are needed. Quantitative analysis of RV function by echocardiography in these patients has been difficult due to complex anatomy and morphology of RV. Conventional 2D echocardiographic measurements, such as TAPSE, manually traced FAC and EF in addition to many different Doppler based measurements have been evaluated as candidates for the quantification of cardiac function in HLHS patients, but unfortunately with poor or no correlation with MRI. The literature has especially focused on manually traced FAC, but as yet there are no studies of intraobserver or interobserver repeatability for this method for assessment of cardiac function in HLHS patients (Altmann 2000, Friedberg 2007b, Friedberg and Silverman 2007, Frommelt 2012, Hughes 2004, Khoo 2011, Petko 2011a, Tham 2014). The 3D echocardiography method is based on the measurement of EF. This is a feasible method and 3D echocardiography EF correlates with MRI EF. However, EF measured by 3D echocardiography is lower than EF measured by MRI, and these measurements cannot be used interchangeably (Bell 2014).

Speckle tracking is an echocardiography-based method, which has been developed for advanced cardiac functional analysis. This method measures myocardial deformation and is independent of the angle of insonation and the geometry of the ventricle. Therefore, it is also suitable for the assessment of RV function in HLHS patients. VVI is a method based on speckle and endocardial contour tracking. Although VVI has been used previously to study patients with HLHS, its use has focused mainly on mechanical

synchrony and segmental function (Bharucha 2013, Friedberg 2007b, Menon 2011, Menon 2011, Menon 2013). However, the relationships of speckle tracking methods, such as VVI, have not been previously validated against a noninvasive golden standard i.e. MRI derived RV-EF in HLHS patients (Bharucha 2013, Friedberg 2007b, Menon 2011, Menon 2013).

We raised the question, about whether different speckle tracking based automated techniques such as QLAB and VVI could be reliably used in clinical practice and repeatable for quantitative assessment of cardiac function in HLHS patients. The functional parameters available for measuring the myocardial function by VVI and used in our study were: EF, FAC, V, S SR. FAC was also measured by QLAB and by manual tracing to study the repeatability between the different programs.

All VVI and QLAB parameters, except myocardial V correlated with MRI EF. In contrast, the correlation was weak for MRI EF for manual FAC. Of all the functional indices measured, both the correlations and multiple regression analysis data support the use of automated FAC as the best echocardiographic predictor of MRI derived RV EF. Intraobserver and interobserver variability was good or excellent for both automated methods (VVI and QLAB), which makes these automated methods feasible options for the assessment of RV function in HLHS at different stages of palliation. This is consistent with previous VVI studies in neonates, infants and children with HLHS before and after second stage palliation (Menon 2011). Importantly, we also found that interobserver repeatability of manually traced FAC was poor. The morphology and geometry of RV in HLHS patients is complex. There is normally increased RV myocardial trabeculation compared to normal LV myocardium. This makes it challenging to track correctly the endomyocardial border in diastole and systole values, on which the measurement of FAC in based. The use of automated techniques make it possible to see whether the tracking is not adequate throughout the whole cardiac cycle and thus allow corrections to be made. In this study, corrections were necessary in most of the cases. Similarly, choosing exactly the correct frame in diastole and systole is challenging and by using automated methods the program automatically select the largest and the smallest areas for analysis. It was a consistent finding in this study that the interobserver variability for manual method was poor.

The correlations between echocardiographic based FAC or EF and MRI based EF did not differ either between the operative stages for any technique. Measuring FAC is suitable for assessing the RV systolic function throughout the operative treatment. Further, there was no difference in the correlations between different morphology groups for manual and VVI-methods, which makes these measurements suitable for all HLHS patients. The QLAB FAC correlation with MRI EF was lower for slit/none LV morphology group than for globular morphology group, which is possibly related to difficulties in tracking in the globular group.

FAC estimates myocardial function from areas in diastole and systole and is

a 2-dimensional parameter. The EF estimates systolic function from the difference in diastole and systole volumes and is a 3-dimensional estimate. When measured from an apical four-chamber view as used here, RV anterior and posterior wall and outflow tract are excluded from the analysis. Therefore, the FAC value can either overestimate or underestimate myocardial function. Further, it is important to keep in mind that EF and FAC measurements are also load and afterload dependent.

Myocardial strain, as used in this study, measures longitudinal deformation of the RV wall. Dominant RV muscle contraction in HLHS patients has been shown to occur mainly in the longitudinal direction (Khoo 2011) and therefore, correlation with MRI EF is good. The same assumptions are made regarding the assessment of SR. After Fontan completion, SR is shown to be a load independent parameter of RV function in HLHS (Schlangen 2014). It is possible that the myocardial S, SR and V measure properties of myocardial function other than EF, which may have a role in the clinical follow-up of HLHS patients, but this has yet to be determined. There are some studies in which myocardial dysfunction in HLHS children was observed earlier when using these parameters as compared to traditional EF (Michel 2016).

Automated methods are more time consuming than the manual approach. In the present study, the time spent for VVI analysis was longest, approximately five minutes, and QLAB analysis was quicker than VVI analysis. More adjustments were needed for VVI analysis. However, the VVI analysis was possible more frequently than the QLAB analysis. This difference might be one explanation for the longer time required for VVI-analysis. Most often tracking in QLAB was not adequate when the left ventricle was globular in morphology, which is often associated with more complex shape of RV in apical four-chamber view. However, time consumed for automated techniques is entirely limited and thereby suitable for clinical practice.

Major limitations for using automated techniques are that some analyzing programs (QLAB) are restricted to specific echocardiographic scanning models and others (VVI) require the transfer of the examination to a specific analysis program. Since different automated methods give different values, we cannot use them interchangeably. Both automated methods used in this study give lower values for FAC than the manual method. This discrepancy is probably related to the fact that in speckle tracking–based automation, the RV border is drawn to the inside of the RV wall, especially in systole. The same phenomenon is seen in EF MRI measurements when comparing semi-automation and manual methods in HLHS patients (Bell 2014). Therefore in clinical practice and for study purposes the same automated technique should be used in serial follow up of HLHS patients. However, it is still unknown, which parameter is the most sensitive to detect true myocardial dysfunction in early phases and which parameter has the best correlation to prognosis of these patients.

6.2 MYOCARDIAL FUNCTION IN HLHS PATIENTS AND PRENATAL DIAGNOSIS

The benefits of a prenatal diagnosis of HLHS on the postnatal RV myocardial function were demonstrated quantitatively in this study. Prenatal diagnosis enables planning of delivery and optimization of postnatal care. All children with prenatal diagnosis in III and IV studies were born in Helsinki University Hospital and transportation distance to the ICU was short. Spontaneous, term delivery was achieved in most pregnancies and there was no difference in gestational age during birth, birth weight or frequency of caesarian section between prenatally and postnatally diagnosed children. Some studies reported that prenatally diagnosed children were delivered earlier than postnatally diagnosed children (Kipps 2011) and this might undo some advantages of prenatal diagnosis, because neonates with younger gestational age at birth more often have difficulties in respiratory adaptation. No routine intubation was needed and prostaglandin treatment for maintaining ductal patency was started immediately after birth in the prenatally born neonates. This results into more stable haemodynamics after birth, which was seen in this and previous studies (Kipps 2011, Kipps 2011, Kumar 1999, Satomi 1999, Sivarajan 2009, Tworetzky 2001). More stable coronary perfusion and efficient systemic circulation were probably reasons for better myocardial function in prenatally diagnosed HLHS patients. In addition, there was a lower incidence of damage to other organs in prenatally diagnosed neonates, which is consistent with the findings of previous studies (Kipps 2011, Kumar 1999, Sivarajan 2009).

Neonates with delayed diagnosis (over 72 hours after birth) tended to have worse myocardial function than their counterparts with early postnatal diagnosis. Some neonates with early postnatal diagnosis had been diagnosed before symptoms (based on oxygen saturation screening or pediatric examination) and therefore, they were in a stable haemodynamic condition, which might be the explanation for this difference. A study by Morris found that the distance from operative hospital had an impact on survival (Morris 2014), but the length of transportation in the present study did not have an impact on myocardial function.

There are conflicting reports in previous studies about the impact of HLHS morphology or LV size on myocardial function or prognosis (Azakie 2001, Gaynor 2002). The HLHS morphology or the size of the aorta found in the present study did not have an effect on myocardial function before stage 1 operation. LV size had a negative correlation to FAC but not to other parameters of myocardial systolic function. This probably represents geometric alterations and not a true difference on myocardial function. The determination of prenatal diagnosis has such a strong impact on myocardial function and this might cover small alterations. It is of note that in the present study, myocardial function analysis was performed after early stabilization at the average age of 2 days. If the VVI measurements had been performed immediately after admission, then the differences between prenatally and postnatally diagnosed infants could very well have been more pronounced.

Birth asphyxia in healthy neonates with no congenital heart defects leads to impaired global left ventricular function (Nestaas 2011, Wei 2009) when evaluated using S and SR analysis. The impact of compromised circulatory and possible coronary perfusion in neonates with HLHS with a single systemic right ventricle is poorly understood during the early transitional period. In the present study, the haemodynamic benefits of prenatal diagnosis on RV function were global (i.e., there was not one area that was more sensitive than any other). Neonates with HLHS have been shown to have mechanical dyssynchrony unrelated to myocardial function (Friedberg 2007a, Moiduddin 2010). Similarly, the present study found that myocardial dysfunction was not associated with mechanical dyssynchrony. The impact of preoperative RV dysfunction on long-term myocardial function after staged single-ventricle palliation requires further exploration.

6.3 MYOCARDIAL FUNCTION IN HLHS PATIENTS DURING THE OPERATIVE PROTOCOL

The initial shunt type selected at stage 1 operation in this study had an impact on later myocardial function. After stage 1 operation HLHS patients with a BT shunt had diminished FAC and larger LV volumes than their counterparts with the RV-PA conduit. No difference was seen in myocardial deformation. More challenging loading conditions and volume load caused by a BT shunt is a probable explanation for this difference in RV volumes as the patients in the BT group also had more frequent tricuspid valve regurgitation. Lower FAC can be related to volume load, because FAC is a load dependent parameter. Further, the difference can reflect more efficient systemic circulation and better coronary perfusion after and RV-PA conduit than the BT shunt, which also has been reported by previous studies (Frommelt 2012, Hughes 2004, Ohye 2010). However, our assumption was that the lower FAC detected in the BT group suggested an impaired systolic function. An alternative explanation may be that a larger volume loaded ventricle may not contract as much as a smaller ventricle to generate the same stroke volume. This could explain the lack of differences in other, perhaps less load-dependent, parameters of function.

After stage 2 palliation, RV size and tricuspid valve insufficiency diminished in both groups and RV systolic function in the BT shunt group recovered, which is consistent with that reported by previous studies (Frommelt 2014, Marx 2013, Ohye 2010). However, the advantage in myocardial function in the RV-PA group after stage 2 and 3 palliations disappeared, which led to lower RV systolic function after stage 3 operation. There are several possible explanations for these changes in the RV-PA group. Creating an RV-PA conduit causes a myocardial scar in the RV free wall. There is local dyskinesia and wall thinning at the site of the scar, which persists even after RV-PA conduit has been taken out (Menon 2011, Menon 2013). Long-term sequelae of the scar might also interfere with global RV function. No difference in mechanical synchrony between shunt types was observed but mechanical dyssynchrony was not related to myocardial function in this

study. Therefore, dyssynchrony is not a probable explanation for diminished RV function after an RV-PA conduit.

Patients with an RV-PA conduit in the present study underwent stage 2 operation at an older age than patients with a BT shunt. A longer time spent in challenging conditions is another possible explanation for diminished myocardial function, but the time spent on stage 1 palliation had no correlation to myocardial function. The RV volumes for both groups diminished after stage 2 operation and there was no difference in RV volumes after stage 2 operation. The volume load during the interstate period between stages 1 and 2, is therefore an unlikely explanation for long-term difference in myocardial function between the two groups.

Shunt type during stage 1 operation had a strong impact on myocardial function during study period and no other factors had a similar correlation with myocardial function during follow up. The HLHS type has been shown to have an impact on myocardial function in HLHS (Murtuza 2012), but this effect was not seen in this study at any stage of palliation. Neither did the residual LV size or the severity of the tricuspid valve insufficiency have any correlation to myocardial function after stage 1 operation.

6.4 MORBIDITY

The operative treatment protocol was the same during the entire study period, except that the shunt type (BT or RV-PA) varied during the study period. Further, contraindications for the operative treatment remained the same. Cardiopulmonary bypass time at stage 1 operation was longer for the RV-PA group than for the BT group, but the cumulative total perfusion time during the whole study period did not differ between shunt types. There was no difference in the number of unintended operations between shunt types. Some studies, however, reported more unintended operations in the RV-PA conduit group, which was mostly related to the PAs (Ohye 2010). This difference was not seen in the present study nor was there a difference in the size of the pulmonary arteries at any palliative stage. This may be related to the surgical approaches. Although patients in the BT group had more significant tricuspid valve regurgitation before stage 2 operation, this difference did not reach statistical significance.

6.5 STRENGTHS AND LIMITATIONS OF THE STUDY

The majority of patients in the prospective part of the study that compared MRI and echocardiographic methods (I, II) had EF in the normal range and there were only a limited number of patients with diminished myocardial function. This finding is indicative of a degree of selection bias, because those patients with impaired myocardial function and poor clinical condition, were not suitable for general anaesthesia, were not included in the study. Most study patients thus had myocardial function in the normal range as

measured by MRI EF. Therefore, the predictive value of VVI will need some further analysis with larger numbers and a wider variability of RV-EF. All echocardiography examinations were performed under general anaesthesia. Without anaesthesia, the quality of echocardiography for VVI analysis could have been lower, which could affect the reliability. However, it is important to perform both MRI and echocardiography in the same clinical and loading conditions, which in this study was during anaesthesia.

In the retrospective myocardial function cohort study (III, IV), the major limitation was the lack of randomized design. However, the study population represents a complete national cohort of HLHS neonates born in Finland between 2003 and 2010. In addition, all patients underwent the entire operative treatment and follow-up in the same institution with 100% follow-up. Four staff surgeons performed all operations, and surgical mortality did not change for any of the stages over the whole study period.

The small size of the study cohort may have limited our ability to detect certain differences between the study groups. Neonates with prenatally chosen compassionate care were not included to analysis, which may have added some selection bias. Another limitation for this study is the non-randomized selection of the shunt type. However, only one shunt type was used during certain time period regardless of clinical or echocardiographic findings, and varied throughout the study period, which limits the selection bias.

6.6 FUTURE DIRECTIONS

This study has raised questions and suggestions for future studies:

-To develop echocardiographic methods that, are not limited to specific apparatus model or program and which can thus be used interchangeably for the assessment of myocardial function in HLHS patients.

-To study the additional risk factors such as *inter alia* genetics for myocardial dysfunction in HLHS patients. Why some patients are more vulnerable to developing myocardial dysfunction during treatment protocol than others?

-To study the impact of different types of surgical methods. For example, should the RV-PA conduit be removed and the ventriculotomy closed at stage 2 operation on myocardial function in HLHS patients?

-To study the impact of the initial shunt type on very long term myocardial function after stage 3 operation.

7 CONCLUSIONS

VVI is suitable for the assessment of RV systolic function in patients with HLHS during different stages of treatment protocol. Reproducibility of all VVI parameters in this study was excellent. All VVI-derived parameters except myocardial V correlate with MRI-derived EF. FAC is the best predictor of MRI-derived EF.

Automation improves the repeatability of echocardiographic measurement of RV systolic function by FAC in patients with HLHS compared to manual tracing of FAC. There were, however, some differences between automated programs in terms of correlation with MRI-derived EF. Limits of agreement between these methods remained wide, which, suggests that different programs cannot be used interchangeably.

A prenatal diagnosis of HLHS improves postnatal RV function and is associated with less metabolic acidosis and less end-organ dysfunction. Prenatal diagnosis of HLHS enables the preplanning for delivery, thus, it provides an opportunity to avoid early hemodynamic pathologies during the fragile transitional period.

The initial shunt type at stage 1 palliation affects RV systolic performance in children with HLHS throughout the subsequent surgical stages of palliation. Following stage 1 palliation, RV FAC was initially lower in infants with a BT shunt, which could reflect either a reduced systolic function or a normalization of function in the context of a larger RV due to volume load. However, a BT shunt at the initial stage was, when assessed after stage 3, associated with a better RV systolic performance in patienst compared to patients with previous RV-PA conduit. Differences in RV systolic performance may be due, at least in part, to a greater time spent prior to stage 2 with volume overload and to the sequale of myocardial scarring in patients that had been initally palliated with an RV-PA conduit. Tha impact of shunt type on subsequent long-term myocardial function needs further investigations along with studies on the impact of myocardial dysfunction at different stages of palliation regarding the long-term prognosis of HLHS patients.

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9 REFERENCES

Altmann K, Printz BF, Solowiejczky DE, Gersony WM, Quaegebeur J, Apfel HD (2000) Two-dimensional echocardiographic assessment of right ventricular function as a predictor of outcome in hypoplastic left heart syndrome. Am J Cardiol 86:964-968

Attar MA, Dechert RE, Schumacher RE (2012) The effect of late preterm birth on mortality of infants with hypoplastic left heart syndrome. Am J Perinatol 29:593-598

Atz AM, Travison TG, Williams IA, Pearson GD, Laussen PC, Mahle WT, Cook AL, Kirsh JA, Sklansky M, Khaikin S, Goldberg C, Frommelt M, Krawczeski C, Puchalski MD, Jacobs JP, Baffa JM, Rychik J, Ohye RG, Pediatric Heart Network Investigators (2010) Prenatal diagnosis and risk factors for preoperative death in neonates with single right ventricle and systemic outflow obstruction: screening data from the Pediatric Heart Network Single Ventricle Reconstruction Trial(*). J Thorac Cardiovasc Surg 140:1245-1250

Avitabile CM, Whitehead K, Fogel M, Mercer-Rosa L (2014) Tricuspid annular plane systolic excursion does not correlate with right ventricular ejection fraction in patients with hypoplastic left heart syndrome after Fontan palliation. Pediatr Cardiol 35:1253-1258

Axt-Fliedner R, Graupner O, Kawecki A, Degenhardt J, Herrmann J, Tenzer A, Doelle A, Willruth A, Steinhard J, Gembruch U, Bahlmann F, Enzensberger C, Fetal Cardiac Imaging Research Group, Germany (2015) Evaluation of right ventricular function in fetuses with hypoplastic left heart syndrome using tissue Doppler techniques. Ultrasound Obstet Gynecol 45:670-677

Azakie T, Merklinger SL, McCrindle BW, Van Arsdell GS, Lee KJ, Benson LN, Coles JG, Williams WG (2001) Evolving strategies and improving outcomes of the modified norwood procedure: a 10-year single-institution experience. Ann Thorac Surg 72:1349-1353

Baba K, Kotani Y, Chetan D, Chaturvedi RR, Lee KJ, Benson LN, Grosse-Wortmann L, Van Arsdell GS, Caldarone CA, Honjo O (2012) Hybrid versus Norwood strategies for single-ventricle palliation. Circulation 126:S123-31

Ballweg JA, Dominguez TE, Ravishankar C, Gaynor JW, Nicolson SC, Spray TL, Tabbutt S (2010) A contemporary comparison of the effect of shunt type in hypoplastic left heart syndrome on the hemodynamics and outcome at Fontan completion. J Thorac Cardiovasc Surg 140:537-544

Ballweg JA, Dominguez TE, Ravishankar C, Kreutzer J, Marino BS, Bird GL, Gruber PJ, Wernovsky G, Gaynor JW, Nicolson SC, Spray TL, Tabbutt S (2007) A contemporary comparison of the effect of shunt type in hypoplastic left heart syndrome on the hemodynamics and outcome at stage 2 reconstruction. J Thorac Cardiovasc Surg 134:297-303

Bautista-Hernandez V, Scheurer M, Thiagarajan R, Salvin J, Pigula FA, Emani S, Fynn-Thompson F, Loyola H, Schiff J, del Nido PJ, Bacha EA (2011) Right ventricle and tricuspid valve function at midterm after the Fontan operation for hypoplastic left heart syndrome: impact of shunt type. Pediatr Cardiol 32:160-166

Bell A, Rawlins D, Bellsham-Revell H, Miller O, Razavi R, Simpson J (2014) Assessment of right ventricular volumes in hypoplastic left heart syndrome by real-time three-dimensional echocardiography: comparison with cardiac magnetic resonance imaging. Eur Heart J Cardiovasc Imaging 15:257-266

Bellsham-Revell HR, Simpson JM, Miller OI, Bell AJ (2013a) Subjective evaluation of right ventricular systolic function in hypoplastic left heart syndrome: how accurate is it?. J Am Soc Echocardiogr 26:52-56

Bellsham-Revell HR, Tibby SM, Bell AJ, Witter T, Simpson J, Beerbaum P, Anderson D, Austin CB, Greil GF, Razavi R (2013b) Serial magnetic resonance imaging in hypoplastic left heart syndrome gives valuable insight into ventricular and vascular adaptation. J Am Coll Cardiol 61:561-570

Bellsham-Revell HR, Tibby SM, Bell AJ, Miller OI, Razavi R, Greil GF, Simpson JM (2012) Tissue Doppler time intervals and derived indices in hypoplastic left heart syndrome. Eur Heart J Cardiovasc Imaging 13:400-407

Bharucha T, Khan R, Mertens L, Friedberg MK (2013) Right ventricular mechanical dyssynchrony and asymmetric contraction in hypoplastic heart syndrome are associated with tricuspid regurgitation. J Am Soc Echocardiogr 26:1214-1220

Bove EL, Ohye RG, Devaney EJ, Hirsch J (2007) Tricuspid valve repair for hypoplastic left heart syndrome and the failing right ventricle. Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu:101-104

Brackley KJ, Kilby MD, Wright JG, Brawn WJ, Sethia B, Stumper O, Holder R, Wyldes MP, Whittle MJ (2000) Outcome after prenatal diagnosis of hypoplastic left-heart syndrome: a case series. Lancet 356:1143-1147

Brooks PA, Khoo NS, Mackie AS, Hornberger LK (2012) Right ventricular function in fetal hypoplastic left heart syndrome. J Am Soc Echocardiogr 25:1068-1074

Brown SB, Raina A, Katz D, Szerlip M, Wiegers SE, Forfia PR (2011) Longitudinal shortening accounts for the majority of right ventricular contraction and improves after pulmonary vasodilator therapy in normal subjects and patients with pulmonary arterial hypertension. Chest 140:27-33

Chetan D, Kotani Y, Jacques F, Poynter JA, Benson LN, Lee KJ, Chaturvedi RR, Friedberg MK, Van Arsdell GS, Caldarone CA, Honjo O (2013) Surgical palliation strategy does not affect interstage ventricular dysfunction or atrioventricular valve regurgitation in children with hypoplastic left heart syndrome and variants. Circulation 128:S205-12

Christensen D, Cardis B, Mahle W, Lewis R, Huckaby J, Favaloro-Sabatier J, Fyfe D (2006) Pre- and postoperative quantitation of right ventricular tissue

Doppler velocities in infants with hypoplastic left heart syndrome. Echocardiography 23:303-307

Fischbach J, Sinzobahamvya N, Haun C, Schindler E, Zartner P, Schneider M, Hraska V, Asfour B, Photiadis J (2013) Interventions after Norwood procedure: comparison of Sano and modified Blalock-Taussig shunt. Pediatr Cardiol 34:112-118

Fixler DE, Nembhard WN, Salemi JL, Ethen MK, Canfield MA (2010) Mortality in first 5 years in infants with functional single ventricle born in Texas, 1996 to 2003. Circulation 121:644-650

Friedberg MK, Silverman NH (2007) The systolic to diastolic duration ratio in children with hypoplastic left heart syndrome: a novel Doppler index of right ventricular function. J Am Soc Echocardiogr 20:749-755

Friedberg MK, Silverman NH, Dubin AM, Rosenthal DN (2007a) Right ventricular mechanical dyssynchrony in children with hypoplastic left heart syndrome. J Am Soc Echocardiogr 20:1073-1079

Friedberg MK, Silverman NH, Dubin AM, Rosenthal DN (2007b) Right ventricular mechanical dyssynchrony in children with hypoplastic left heart syndrome. J Am Soc Echocardiogr 20:1073-1079

Friedman KG, Salvin JW, Wypij D, Gurmu Y, Bacha EA, Brown DW, Laussen PC, Scheurer MA (2011) Risk factors for failed staged palliation after bidirectional Glenn in infants who have undergone stage one palliation. Eur J Cardiothorac Surg 40:1000-1006

Frommelt PC, Gerstenberger E, Cnota JF, Cohen MS, Gorentz J, Hill KD, John JB, Levine JC, Lu J, Mahle WT, McCandless RT, Mertens L, Pearson GD, Spencer C, Thacker D, Williams IA, Wong PC, Newburger JW, Pediatric Heart Network Investigators (2014) Impact of initial shunt type on cardiac size and function in children with single right ventricle anomalies before the Fontan procedure: the single ventricle reconstruction extension trial. J Am Coll Cardiol 64:2026-2035

Frommelt PC, Guey LT, Minich LL, Bhat M, Bradley TJ, Colan SD, Ensing G, Gorentz J, Heydarian H, John JB, Lai WW, Levine JC, Mahle WT, Miller SG, Ohye RG, Pearson GD, Shirali GS, Wong PC, Cohen MS, Pediatric Heart Network Investigators (2012) Does initial shunt type for the Norwood procedure affect echocardiographic measures of cardiac size and function during infancy?: the Single Vventricle Reconstruction trial. Circulation 125:2630-2638

Galindo A, Nieto O, Villagra S, Graneras A, Herraiz I, Mendoza A (2009) Hypoplastic left heart syndrome diagnosed in fetal life: associated findings, pregnancy outcome and results of palliative surgery. Ultrasound Obstet Gynecol 33:560-566

Gaynor JW, Mahle WT, Cohen MI, Ittenbach RF, DeCampli WM, Steven JM, Nicolson SC, Spray TL (2002) Risk factors for mortality after the Norwood procedure. Eur J Cardiothorac Surg 22:82-89

Gelehrter S, Fifer CG, Armstrong A, Hirsch J, Gajarski R (2011) Outcomes of hypoplastic left heart syndrome in low-birth-weight patients. Pediatr Cardiol 32:1175-1181

Ghanayem NS, Allen KR, Tabbutt S, Atz AM, Clabby ML, Cooper DS, Eghtesady P, Frommelt PC, Gruber PJ, Hill KD, Kaltman JR, Laussen PC, Lewis AB, Lurito KJ, Minich LL, Ohye RG, Schonbeck JV, Schwartz SM, Singh RK, Goldberg CS, Pediatric Heart Network Investigators (2012) Interstage mortality after the Norwood procedure: Results of the multicenter Single Ventricle Reconstruction trial. J Thorac Cardiovasc Surg 144:896-906

Gibbs JL, Wren C, Watterson KG, Hunter S, Hamilton JR (1993) Stenting of the arterial duct combined with banding of the pulmonary arteries and atrial septectomy or septostomy: a new approach to palliation for the hypoplastic left heart syndrome. Br Heart J 69:551-555

Gokhale J, Husain N, Nicholson L, Texter KM, Zaidi AN, Cua CL (2013) QRS duration and mechanical dyssynchrony correlations with right ventricular function after fontan procedure. J Am Soc Echocardiogr 26:154-159

Graham EM, Zyblewski SC, Phillips JW, Shirali GS, Bradley SM, Forbus GA, Bandisode VM, Atz AM (2010) Comparison of Norwood shunt types: do the outcomes differ 6 years later?. Ann Thorac Surg 90:31-35

Graham EM, Atz AM, Bradley SM, Scheurer MA, Bandisode VM, Laudito A, Shirali GS (2007) Does a ventriculotomy have deleterious effects following palliation in the Norwood procedure using a shunt placed from the right ventricle to the pulmonary arteries?. Cardiol Young 17:145-150

Graupner O, Enzensberger C, Wieg L, Willruth A, Steinhard J, Gembruch U, Doelle A, Bahlmann F, Kawecki A, Degenhardt J, Wolter A, Herrmann J, Axt-Fliedner R, Fetal Cardiac Imaging Research Group Germany (2016) Evaluation of right ventricular function in fetal hypoplastic left heart syndrome by color tissue Doppler imaging. Ultrasound Obstet Gynecol 47:732-738

Hammarstrom E, Wranne B, Pinto FJ, Puryear J, Popp RL (1991) Tricuspid annular motion. J Am Soc Echocardiogr 4:131-139

Hill GD, Frommelt PC, Stelter J, Campbell MJ, Cohen MS, Kharouf R, Lai WW, Levine JC, Lu JC, Menon SC, Slesnick TC, Wong PC, Saudek DE (2015) Impact of initial norwood shunt type on right ventricular deformation: the single ventricle reconstruction trial. J Am Soc Echocardiogr 28:517-521

Hinton RB, Martin LJ, Rame-Gowda S, Tabangin ME, Cripe LH, Benson DW (2009) Hypoplastic left heart syndrome links to chromosomes 10q and 6q and is genetically related to bicuspid aortic valve. J Am Coll Cardiol 53:1065-1071

Hinton RB,Jr, Martin LJ, Tabangin ME, Mazwi ML, Cripe LH, Benson DW (2007) Hypoplastic left heart syndrome is heritable. J Am Coll Cardiol 50:1590-1595

Hornberger LK, Sanders SP, Rein AJ, Spevak PJ, Parness IA, Colan SD (1995) Left heart obstructive lesions and left ventricular growth in the midtrimester fetus. A longitudinal study. Circulation 92:1531-1538

Hughes ML, Tsang VT, Kostolny M, Giardini A, Muthurangu V, Taylor AM, Brown K (2011) Lessons from inter-stage cardiac magnetic resonance imaging in predicting survival for patients with hypoplastic left heart syndrome. Cardiol Young 21:646-653

Hughes ML, Shekerdemian LS, Brizard CP, Penny DJ (2004) Improved early ventricular performance with a right ventricle to pulmonary artery conduit in stage 1 palliation for hypoplastic left heart syndrome: evidence from strain Doppler echocardiography. Heart 90:191-194

Husain N, Gokhale J, Nicholson L, Cheatham JP, Holzer RJ, Cua CL (2013) Noninvasive estimation of ventricular filling pressures in patients with single right ventricles. J Am Soc Echocardiogr 26:1330-1336

Januszewska K, Stebel A, Malec E (2007) Consequences of right ventricle-to-pulmonary artery shunt at the first stage for the Fontan operation. Ann Thorac Surg 84:1611-1617

Jean-St-Michel E, Chetan D, Schwartz SM, Van Arsdell GS, Floh AA, Honjo O, Conway J (2016) Outcomes in Patients with Persistent Ventricular Dysfunction After Stage I Palliation for Hypoplastic Left Heart Syndrome. Pediatr Cardiol 37:239-247

Jiang Y, Xu Y, Tang J, Xia H (2016) Assessment of Structural and Functional Abnormalities of the Myocardium and the Ascending Aorta in Fetus with Hypoplastic Left Heart Syndrome. Biomed Res Int 2016:2616729

Kasnar-Samprec J, Kuhn A, Horer J, Vogt M, Cleuziou J, Lange R, Schreiber C (2012) Unloading of right ventricle by bidirectional superior cavopulmonary anastomosis in hypoplastic left heart syndrome patients promotes remodeling of systemic right ventricle but does not improve tricuspid regurgitation. J Thorac Cardiovasc Surg 144:1102-1108

Khoo NS, Smallhorn JF, Kaneko S, Myers K, Kutty S, Tham EB (2011) Novel insights into RV adaptation and function in hypoplastic left heart syndrome between the first 2 stages of surgical palliation. JACC Cardiovasc Imaging 4:128-137

Kimball TR, Witt SA, Khoury PR, Daniels SR (1996) Automated echocardiographic analysis of systemic ventricular performance in hypoplastic left heart syndrome. J Am Soc Echocardiogr 9:629-636

Kipps AK, Feuille C, Azakie A, Hoffman JI, Tabbutt S, Brook MM, Moon-Grady AJ (2011) Prenatal diagnosis of hypoplastic left heart syndrome in current era. Am J Cardiol 108:421-427

Knirsch W, Liamlahi R, Hug MI, Hoop R, von Rhein M, Pretre R, Kretschmar O, Latal B (2012) Mortality and neurodevelopmental outcome at 1 year of age comparing hybrid and Norwood procedures. Eur J Cardiothorac Surg 42:33-39

Kotani Y, Kasahara S, Fujii Y, Yoshizumi K, Oshima Y, Otsuki S, Akagi T, Sano S (2009) Clinical outcome of the Fontan operation in patients with impaired ventricular function. Eur J Cardiothorac Surg 36:683-687

Kumar RK, Newburger JW, Gauvreau K, Kamenir SA, Hornberger LK (1999) Comparison of outcome when hypoplastic left heart syndrome and transposition of the great arteries are diagnosed prenatally versus when diagnosis of these two conditions is made only postnatally. Am J Cardiol 83:1649-1653

Lai L, Laussen PC, Cua CL, Wessel DL, Costello JM, del Nido PJ, Mayer JE, Thiagarajan RR (2007) Outcomes after bidirectional Glenn operation: Blalock-Taussig shunt versus right ventricle-to-pulmonary artery conduit. Ann Thorac Surg 83:1768-1773

Leirgul E, Fomina T, Brodwall K, Greve G, Holmstrom H, Vollset SE, Tell GS, Oyen N (2014) Birth prevalence of congenital heart defects in Norway 1994-2009--a nationwide study. Am Heart J 168:956-964

Maher KO, Pizarro C, Gidding SS, Januszewska K, Malec E, Norwood WI,Jr, Murphy JD (2003) Hemodynamic profile after the Norwood procedure with right ventricle to pulmonary artery conduit. Circulation 108:782-784

Mahle WT, Cuadrado AR, Tam VK (2003) Early experience with a modified Norwood procedure using right ventricle to pulmonary artery conduit. Ann Thorac Surg 76:1084-8; discussion 1089

Mahle WT, Clancy RR, McGaurn SP, Goin JE, Clark BJ (2001a) Impact of prenatal diagnosis on survival and early neurologic morbidity in neonates with the hypoplastic left heart syndrome. Pediatrics 107:1277-1282

Mahle WT, Clancy RR, McGaurn SP, Goin JE, Clark BJ (2001b) Impact of prenatal diagnosis on survival and early neurologic morbidity in neonates with the hypoplastic left heart syndrome. Pediatrics 107:1277-1282

Mahle WT, Spray TL, Wernovsky G, Gaynor JW, Clark BJ,3rd (2000) Survival after reconstructive surgery for hypoplastic left heart syndrome: A 15-year experience from a single institution. Circulation 102:III136-41

Mair R, Tulzer G, Sames E, Gitter R, Lechner E, Steiner J, Hofer A, Geiselseder G, Gross C (2003) Right ventricular to pulmonary artery conduit instead of modified Blalock-Taussig shunt improves postoperative hemodynamics in newborns after the Norwood operation. J Thorac Cardiovasc Surg 126:1378-1384

Makikallio K, McElhinney DB, Levine JC, Marx GR, Colan SD, Marshall AC, Lock JE, Marcus EN, Tworetzky W (2006) Fetal aortic valve stenosis and the evolution of hypoplastic left heart syndrome: patient selection for fetal intervention. Circulation 113:1401-1405

Malec E, Januszewska K, Kolcz J, Mroczek T (2003) Right ventricle-to-pulmonary artery shunt versus modified Blalock-Taussig shunt in the Norwood procedure for hypoplastic left heart syndrome - influence on early and late haemodynamic status. Eur J Cardiothorac Surg 23:728-33; discussion 733-4

Marshall AC, Tworetzky W, Bergersen L, McElhinney DB, Benson CB, Jennings RW, Wilkins-Haug LE, Marx GR, Lock JE (2005) Aortic

valvuloplasty in the fetus: technical characteristics of successful balloon dilation. J Pediatr 147:535-539

Marx GR, Shirali G, Levine JC, Guey LT, Cnota JF, Baffa JM, Border WL, Colan S, Ensing G, Friedberg MK, Goldberg DJ, Idriss SF, John JB, Lai WW, Lu M, Menon SC, Ohye RG, Saudek D, Wong PC, Pearson GD, Pediatric Heart Network Investigators (2013) Multicenter study comparing shunt type in the norwood procedure for single-ventricle lesions: three-dimensional echocardiographic analysis. Circ Cardiovasc Imaging 6:934-942

Menon SC, Erickson LK, McFadden M, Miller DV (2013) Effect of ventriculotomy on right-ventricular remodeling in hypoplastic left heart syndrome: a histopathological and echocardiography correlation study. Pediatr Cardiol 34:354-363

Menon SC, Minich LL, Casper TC, Puchalski MD, Hawkins JA, Tani LY (2011) Regional myocardial dysfunction following Norwood with right ventricle to pulmonary artery conduit in patients with hypoplastic left heart syndrome. J Am Soc Echocardiogr 24:826-833

Mertens LL, Friedberg MK (2010) Imaging the right ventricle--current state of the art. Nat Rev Cardiol 7:551-563

Michel M, Logoteta J, Entenmann A, Hansen JH, Voges I, Kramer HH, Petko C (2016) Decline of Systolic and Diastolic 2D Strain Rate During Follow-Up of HLHS Patients After Fontan Palliation. Pediatr Cardiol

Michelfelder EC, Vermilion RP, Ludomirsky A, Beekman RH, Lloyd TR (1996) Comparison of simultaneous Doppler- and catheter-derived right ventricular dP/dt in hypoplastic left heart syndrome. Am J Cardiol 77:212-214

Miller TA, Puchalski MD, Weng C, Menon SC (2012) Regional and global myocardial deformation of the fetal right ventricle in hypoplastic left heart syndrome. Prenat Diagn 32:949-953

Moiduddin N, Texter KM, Zaidi AN, Hershenson JA, Stefaniak C, Hayes J, Cua CL (2010) Two-dimensional speckle strain and dyssynchrony in single left ventricles vs. normal left ventricles. Congenit Heart Dis 5:579-586

Morris CD, Outcalt J, Menashe VD (1990) Hypoplastic left heart syndrome: natural history in a geographically defined population. Pediatrics 85:977-983

Morris SA, Ethen MK, Penny DJ, Canfield MA, Minard CG, Fixler DE, Nembhard WN (2014) Prenatal diagnosis, birth location, surgical center, and neonatal mortality in infants with hypoplastic left heart syndrome. Circulation 129:285-292

Motonaga KS, Miyake CY, Punn R, Rosenthal DN, Dubin AM (2012) Insights into dyssynchrony in hypoplastic left heart syndrome. Heart Rhythm 9:2010-2015

Murtuza B, Stumper O, Wall D, Stickley J, Jones TJ, Barron DJ, Brawn WJ (2012) The effect of morphologic subtype on outcomes following the Sano-Norwood procedure. Eur J Cardiothorac Surg 42:787-793

Muthurangu V, Taylor AM, Hegde SR, Johnson R, Tulloh R, Simpson JM, Qureshi S, Rosenthal E, Baker E, Anderson D, Razavi R (2005) Cardiac magnetic resonance imaging after stage I Norwood operation for hypoplastic left heart syndrome. Circulation 112:3256-3263

Nadorlik H, Fleishman C, Brown DW, Miller-Tate H, Lenahan P, Nicholson L, Wheller J, Cua CL (2014) Survey of How Pediatric Cardiologists Noninvasively Evaluate Patients with Hypoplastic Left Heart Syndrome. Congenit Heart Dis

Nestaas E, Stoylen A, Brunvand L, Fugelseth D (2011) Longitudinal strain and strain rate by tissue Doppler are more sensitive indices than fractional shortening for assessing the reduced myocardial function in asphyxiated neonates. Cardiol Young 21:1-7

Norwood WI,Jr, Jacobs ML, Murphy JD (1992) Fontan procedure for hypoplastic left heart syndrome. Ann Thorac Surg 54:1025-9; discussion 1029-30

O'Connor MJ, Elias MD, Cohen MS, Quartermain MD (2012) Outcomes of infants undergoing superior cavopulmonary connection in the presence of ventricular dysfunction. Pediatr Cardiol 33:547-553

Ohye RG, Sleeper LA, Mahony L, Newburger JW, Pearson GD, Lu M, Goldberg CS, Tabbutt S, Frommelt PC, Ghanayem NS, Laussen PC, Rhodes JF, Lewis AB, Mital S, Ravishankar C, Williams IA, Dunbar-Masterson C, Atz AM, Colan S, Minich LL, Pizarro C, Kanter KR, Jaggers J, Jacobs JP, Krawczeski CD, Pike N, McCrindle BW, Virzi L, Gaynor JW, Pediatric Heart Network Investigators (2010) Comparison of shunt types in the Norwood procedure for single-ventricle lesions. N Engl J Med 362:1980-1992

Ohye RG, Ludomirsky A, Devaney EJ, Bove EL (2004) Comparison of right ventricle to pulmonary artery conduit and modified Blalock-Taussig shunt hemodynamics after the Norwood operation. Ann Thorac Surg 78:1090-1093

Padalino MA, Castellani C, Toffoli S, Della Barbera M, Milanesi O, Thiene G, Stellin G, Angelini A (2008) Pathological changes and myocardial remodelling related to the mode of shunting following surgical palliation for hypoplastic left heart syndrome. Cardiol Young 18:415-422

Petko C, Uebing A, Furck A, Rickers C, Scheewe J, Kramer HH (2011a) Changes of right ventricular function and longitudinal deformation in children with hypoplastic left heart syndrome before and after the Norwood operation. J Am Soc Echocardiogr 24:1226-1232

Petko C, Voges I, Schlangen J, Scheewe J, Kramer HH, Uebing AS (2011b) Comparison of right ventricular deformation and dyssynchrony in patients with different subtypes of hypoplastic left heart syndrome after Fontan surgery using two-dimensional speckle tracking. Cardiol Young 21:677-683

Petko C, Hoffmann U, Moller P, Scheewe J, Kramer HH, Uebing A (2010) Assessment of ventricular function and dyssynchrony before and after stage 2 palliation of hypoplastic left heart syndrome using two-dimensional speckle tracking. Pediatr Cardiol 31:1037-1042

Photiadis J, Sinzobahamvya N, Haun C, Schneider M, Zartner P, Schindler E, Asfour B, Hraska V (2012) Does the shunt type determine mid-term outcome after Norwood operation?. Eur J Cardiothorac Surg 42:209-15; discussion 215-6

Photiadis J, Urban AE, Sinzobahamvya N, Fink C, Schindler E, Schneider M, Marie Brecher A, Asfour B (2005) Restrictive left atrial outflow adversely affects outcome after the modified Norwood procedure. Eur J Cardiothorac Surg 27:962-967

Pirat B, McCulloch ML, Zoghbi WA (2006) Evaluation of global and regional right ventricular systolic function in patients with pulmonary hypertension using a novel speckle tracking method. Am J Cardiol 98:699-704

Pizarro C, Mroczek T, Malec E, Norwood WI (2004) Right ventricle to pulmonary artery conduit reduces interim mortality after stage 1 Norwood for hypoplastic left heart syndrome. Ann Thorac Surg 78:1959-63; discussion 1963-4

Pizarro C, Malec E, Maher KO, Januszewska K, Gidding SS, Murdison KA, Baffa JM, Norwood WI (2003) Right ventricle to pulmonary artery conduit improves outcome after stage I Norwood for hypoplastic left heart syndrome. Circulation 108 Suppl 1:II155-60

Polimenakos AC, Bokowski JW, Ghawi HS, El-Zein CF, Ilbawi MN (2014) Fate of ventricular and valve performance following early bidirectional Glenn procedure after Norwood operation controlled for hypoplastic left heart syndome anatomic subtype. Pediatr Cardiol 35:332-343

Saiki H, Kuwata S, Kurishima C, Masutani S, Senzaki H (2016) Vulnerability of Coronary Circulation After Norwood Operation. Ann Thorac Surg 101:1544-1551

Salih C, McCarthy KP, Ho SY (2004) The fibrous matrix of ventricular myocardium in hypoplastic left heart syndrome: a quantitative and qualitative analysis. Ann Thorac Surg 77:36-40

Salminen J et Sairanen H. (2017) Synnynnäisten sydänvikojen leikkaushoito. In the book Käytännön Lastenkardiologiaa. Ojala T, Happinen J-M, Jokinen E, Pihkala J. Duodecim 2017:140-150.

Sano S, Huang SC, Kasahara S, Yoshizumi K, Kotani Y, Ishino K (2009) Risk factors for mortality after the Norwood procedure using right ventricle to pulmonary artery shunt. Ann Thorac Surg 87:178-85; discussion 185-6

Sano S, Ishino K, Kado H, Shiokawa Y, Sakamoto K, Yokota M, Kawada M (2004) Outcome of right ventricle-to-pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome: a multi-institutional study. Ann Thorac Surg 78:1951-7; discussion 1957-8

Sano S, Ishino K, Kawada M, Arai S, Kasahara S, Asai T, Masuda Z, Takeuchi M, Ohtsuki S (2003) Right ventricle-pulmonary artery shunt in first-stage palliation of hypoplastic left heart syndrome. J Thorac Cardiovasc Surg 126:504-9; discussion 509-10

Sarkola T. (2017) Yksikammioiset sydänviat. In the book Käytännön Lastenkardiologiaa. Ojala T, Happinen J-M, Jokinen E, Pihkala J. Duodecim 2017:121-8.

Satomi G, Yasukochi S, Shimizu T, Takigiku K, Ishii T (1999) Has fetal echocardiography improved the prognosis of congenital heart disease? Comparison of patients with hypoplastic left heart syndrome with and without prenatal diagnosis. Pediatr Int 41:728-732

Scheurer MA, Salvin JW, Vida VL, Fynn-Thompson F, Bacha EA, Pigula FA, Mayer JE,Jr, del Nido PJ, Wessel DL, Laussen PC, Thiagarajan RR (2008) Survival and clinical course at Fontan after stage one palliation with either a modified Blalock-Taussig shunt or a right ventricle to pulmonary artery conduit. J Am Coll Cardiol 52:52-59

Schlangen J, Petko C, Hansen JH, Michel M, Hart C, Uebing A, Fischer G, Becker K, Kramer HH (2014) Two-dimensional global longitudinal strain rate is a preload independent index of systemic right ventricular contractility in hypoplastic left heart syndrome patients after Fontan operation. Circ Cardiovasc Imaging 7:880-886

Seliem MA, Baffa JM, Vetter JM, Chen SL, Chin AJ, Norwood WI,Jr (1993) Changes in right ventricular geometry and heart rate early after hemi-Fontan procedure. Ann Thorac Surg 55:1508-1512

Shimada S, Robles C, Illigens BM, Casar Berazaluce AM, del Nido PJ, Friehs I (2015) Distention of the Immature Left Ventricle Triggers Development of Endocardial Fibroelastosis: An Animal Model of Endocardial Fibroelastosis Introducing Morphopathological Features of Evolving Fetal Hypoplastic Left Heart Syndrome. Biomed Res Int 2015:462469

Silva JP, Fonseca L, Baumgratz JF, Castro RM, Franchi SM, Sylos C, Grassi LM, Cattani CA, Lopes LM, Vila JH (2007) Hypoplastic left heart syndrome: the influence of surgical strategy on outcomes. Arq Bras Cardiol 88:354-360

Simsic JM, Bradley SM, Stroud MR, Atz AM (2005) Risk factors for interstage death after the Norwood procedure. Pediatr Cardiol 26:400-403

Sivarajan V, Penny DJ, Filan P, Brizard C, Shekerdemian LS (2009) Impact of antenatal diagnosis of hypoplastic left heart syndrome on the clinical presentation and surgical outcomes: the Australian experience. J Paediatr Child Health 45:112-117

Stamm C, Anderson RH, Ho SY (1997) The morphologically tricuspid valve in hypoplastic left heart syndrome. Eur J Cardiothorac Surg 12:587-592

Stiver C, Nadorlik H, Nicholson L, Janevski I, Belfrage K, Wheller J, Cua CL (2015) Diastolic Dyssynchrony Differences in Patients with Single Right Ventricles vs. Control Patients. Congenit Heart Dis 10:326-332

Szwast A, Tian Z, McCann M, Donaghue D, Rychik J (2009) Right ventricular performance in the fetus with hypoplastic left heart syndrome. Ann Thorac Surg 87:1214-1219

Tabbutt S, Dominguez TE, Ravishankar C, Marino BS, Gruber PJ, Wernovsky G, Gaynor JW, Nicolson SC, Spray TL (2005) Outcomes after

the stage I reconstruction comparing the right ventricular to pulmonary artery conduit with the modified Blalock Taussig shunt. Ann Thorac Surg 80:1582-90; discussion 1590-1

Thakur V, Munk N, Mertens L, Nield LE (2016) Does prenatal diagnosis of hypoplastic left heart syndrome make a difference? - A systematic review. Prenat Diagn 36:854-863

Tham EB, Smallhorn JF, Kaneko S, Valiani S, Myers KA, Colen TM, Kutty S, Khoo NS (2014) Insights into the evolution of myocardial dysfunction in the functionally single right ventricle between staged palliations using speckle-tracking echocardiography. J Am Soc Echocardiogr 27:314-322

Tibballs J, Cantwell-Bartl A (2008) Outcomes of management decisions by parents for their infants with hypoplastic left heart syndrome born with and without a prenatal diagnosis. J Paediatr Child Health 44:321-324

Tibballs J, Kawahira Y, Carter BG, Donath S, Brizard C, Wilkinson J (2007) Outcomes of surgical treatment of infants with hypoplastic left heart syndrome: an institutional experience 1983-2004. J Paediatr Child Health 43:746-751

Trowitzsch E, Colan SD, Sanders SP (1985) Two-dimensional echocardiographic estimation of right ventricular area change and ejection fraction in infants with systemic right ventricle (transposition of the great arteries or hypoplastic left heart syndrome). Am J Cardiol 55:1153-1157

Tweddell JS, Sleeper LA, Ohye RG, Williams IA, Mahony L, Pizarro C, Pemberton VL, Frommelt PC, Bradley SM, Cnota JF, Hirsch J, Kirshbom PM, Li JS, Pike N, Puchalski M, Ravishankar C, Jacobs JP, Laussen PC, McCrindle BW, Pediatric Heart Network Investigators (2012) Intermediate-term mortality and cardiac transplantation in infants with single-ventricle lesions: risk factors and their interaction with shunt type. J Thorac Cardiovasc Surg 144:152-159

Tweddell JS, Hoffman GM, Mussatto KA, Fedderly RT, Berger S, Jaquiss RD, Ghanayem NS, Frisbee SJ, Litwin SB (2002) Improved survival of patients undergoing palliation of hypoplastic left heart syndrome: lessons learned from 115 consecutive patients. Circulation 106:I82-9

Tworetzky W, Wilkins-Haug L, Jennings RW, van der Velde ME, Marshall AC, Marx GR, Colan SD, Benson CB, Lock JE, Perry SB (2004) Balloon dilation of severe aortic stenosis in the fetus: potential for prevention of hypoplastic left heart syndrome: candidate selection, technique, and results of successful intervention. Circulation 110:2125-2131

Tworetzky W, McElhinney DB, Reddy VM, Brook MM, Hanley FL, Silverman NH (2001) Improved surgical outcome after fetal diagnosis of hypoplastic left heart syndrome. Circulation 103:1269-1273

Vyas HV, Eidem BW, Cetta F, Acharya G, Huhta J, Roberson D, Cuneo B (2011) Myocardial tissue Doppler velocities in fetuses with hypoplastic left heart syndrome. Ann Pediatr Cardiol 4:129-134

Walsh MA, McCrindle BW, Dipchand A, Manlhiot C, Hickey E, Caldarone CA, Van Arsdell GS, Schwartz SM (2009) Left ventricular morphology influences mortality after the Norwood operation. Heart 95:1238-1244

Watanabe E, Arakawa T, Uchiyama T, Tong M, Yasui K, Takeuchi H, Terasawa T, Kodama I, Hishida H (2007) Prognostic significance of circadian variability of RR and QT intervals and QT dynamicity in patients with chronic heart failure. Heart Rhythm 4:999-1005

Wei Y, Xu J, Xu T, Fan J, Tao S (2009) Left ventricular systolic function of newborns with asphyxia evaluated by tissue Doppler imaging. Pediatr Cardiol 30:741-746

Wilder TJ, McCrindle BW, Phillips AB, Blackstone EH, Rajeswaran J, Williams WG, DeCampli WM, Jacobs JP, Jacobs ML, Karamlou T, Kirshbom PM, Lofland GK, Ziemer G, Hickey EJ (2015) Survival and right ventricular performance for matched children after stage-1 Norwood: Modified Blalock-Taussig shunt versus right-ventricle-to-pulmonary-artery conduit. J Thorac Cardiovasc Surg

Wisler J, Khoury PR, Kimball TR (2008) The effect of left ventricular size on right ventricular hemodynamics in pediatric survivors with hypoplastic left heart syndrome. J Am Soc Echocardiogr 21:464-469